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THE EFFECTS UPON THE pH OF DOG SERUM AND URINE OF THE ALPHA AND BETA FRACTIONS OF POSTERIOR LOBE PITUITARY EXTRACT

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The effects of the injection of the alpha and beta fractions of posterior-lobe pituitary extracts upon the CO_2 tension of the blood of unanesthetized dogs has been noted previously, for example, by Geiling, De Lawder, and Rosenfeld (1931) who found that samples of venous blood when collected under local anesthesia from trained dogs underwent marked changes in color as a result of the injection of "Pitocin" or "Pitressin." The alpha fraction caused an increase in the CO_2 tension, the beta a fall. Poulsson (1930) reported that human urine became more alkaline as the result of the subcutaneous injection of "Pituitrin." It seemed desirable to conduct a series of experiments to correlate these findings and to determine if possible which of the two fractions is responsible for the change in the acidity of the urine. This paper presents data from such experiments.

METHOD. *Collection of samples.* Normal healthy dogs were used. Samples of blood were obtained from an external jugular vein and transferred to tonometers without loss of CO_2 as described in another place (McIntyre, 1932). To avoid possible effects due to the ingestion of food, samples were always taken approximately eighteen hours after the animals' feeding time. No form of anesthesia was used. Urine was obtained by catheterization. Injections were usually made subcutaneously in amounts of 0.5 unit per kilogram of the pressor and oxytocic fractions respectively.[1] In a few experiments the injections were made intravenously in somewhat smaller amounts.

Measurement of pH. Measurements of pH were accomplished with the aid of a Morton-type high conductivity glass electrode used in connection

[1] The author is indebted to Dr. Oliver Kamm of the Parke-Davis Laboratory for supplies of Pitocin and Pitressin used throughout these experiments.

with a circuit incorporating a "Plitron" thermionic valve. This valve has been used previously in biological investigation (Hill, 1931) (van Dyke and Bennett, 1932). The circuit employed by the author somewhat resembles one described by the General Electric Company (1932) but differs from earlier circuits used with this valve inasmuch as switching devices in the control grid lead are avoided, thus eliminating a frequent source of trouble. This is not the place to furnish a complete description of the construction and operation of the apparatus; however, it may be stated that the arrangement shown in the accompanying diagram was found to have very marked stability and ease of operation and to be capable of giving rapid readings.

Accuracy of the method. While changes in the control grid input corresponding to pH 0.001 caused deflections of the galvanometer image of

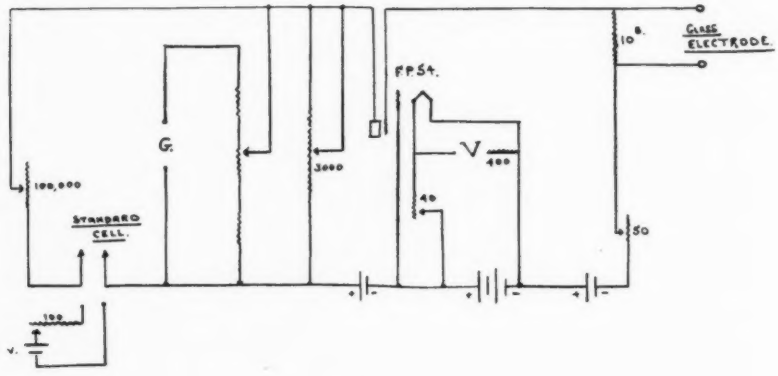

Fig. 1

approximately 15 mm. such deflections were not consistently reproducible. Consistent and easily reproducible deflections were produced by increments in pH of 0.005 consequently the apparatus may be used with considerable confidence for the measurement of changes in pH of 0.01. No attempt was made to control the temperature of either the serum or urine at the time of measurement which was done at room temperature; however, the temperature was never lower than 17° nor higher than 22° during readings.

RESULTS. In a series of 6 experiments in which samples of blood were obtained from various animals under experimental conditions but without the injection of either extract it was found that the pH of the serum sometimes showed spontaneous variations. As a rule however in animals that remained quiet and were not excited by the removal of blood the changes in

pH were smaller than the experimental error of the method. In 5 of 7 animals injected subcutaneously with the alpha fraction (Pitocin) there was a small but definite increase in the H ion concentration of the serum. With the beta fraction (Pitressin) there was either no change (4 experiments) or a slight decrease in H ion concentration in the serum (4 experiments) and in no instance was there an increase. The changes in the urine were most striking. In every case there was a marked decrease in the H ion concentration following the injection of either the alpha or beta fraction and irrespective of any effect upon the serum. The changes in urine pH ranged from a greatest with Pitressin (4.46 to 7.32) to a least with Pitocin

TABLE 1

EXPERIMENT NUMBER	SERUM (i)	SERUM (ii)	URINE (i)	URINE (ii)
		Pitocin		
1	7.39	7.37	5.82	6.82
2	7.41	7.38	6.21	7.74
3	7.37	7.37	5.64	7.62
4	7.37	7.37	5.21	7.01
5	7.40	7.38	5.12	5.30
6	7.35	7.35	5.70	6.94
7	7.36	7.33	4.99	7.31
		Pitressin		
1	7.38	7.38	4.98	7.22
2	7.40	7.40	4.61	7.08
3	7.40	7.40	5.37	6.79
4	7.39	7.41	5.01	7.36
5	7.41	7.42	5.13	7.22
6	7.37	7.40	5.22	6.94
7	7.36	7.40	4.46	7.32
8	7.42	7.42	4.94	7.30

(5.12 to 5.30). The accompanying table contains data from 7 experiments with "Pitocin" and 8 experiments with "Pitressin" and is self-explanatory.

DISCUSSION. The observations of Geiling and co-workers (1931) upon the changes in blood induced by the injection of the alpha and beta posterior-lobe fractions into unanesthetized animals were easy of confirmation. While the shifts in hydrogen ion concentration as far as serum was concerned were not large, the changes in the appearance of the whole blood were very striking and not infrequently it was possible to decide without hesitation as to which of the two fractions had been injected. In view of these marked differences in the effects of the two fractions upon the blood it is surprising to find that following the injection of either fraction the

urine becomes markedly alkaline. The question at once arises as to the amount of one fraction present in the other as an impurity. The manufacturer stated that there were 0.2 unit of pressor substance in 10 units of the oxytocic material and 0.3 unit of the oxytocic material in 10 units of the pressor substance. While no evidence is here presented proving that the effect upon the urine is not due to one of the fractions alone, such an explanation in view of their opposite effects upon the serum appears somewhat unlikely, but further experimentation must await the production of pure fractions before this question can be finally answered.

SUMMARY AND CONCLUSION

Experiments are described in which the effects of the alpha and beta fractions of posterior-lobe pituitary extracts upon the pH of the serum and urine of normal unanesthetized dogs were examined. The pH was measured electrometrically with the aid of a Morton glass electrode and an amplifying circuit. It is concluded in the normal dog that has fasted eighteen hours that:

1. Pitressin injected subcutaneously appears to be capable of slightly decreasing the H ion concentration of serum.

2. Pitocin injected subcutaneously appears to have slight but opposite effects on the H ion concentration.

3. Either of these fractions in its present state of purity causes a definite increase in the alkalinity of the urine irrespective of its effect, if any, upon the serum.

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A STUDY OF THE METABOLIC ACTIVITY OF THE PANCREAS

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One of us (E. U. S.) has been interested in the problem of the isolation of, and the study of the physiological and chemical properties of pancreatic secretin. He has been able to make a preparation of relatively high purity. This material will, in minute doses, cause an abundant secretion of pancreatic juice and a small secretion of bile without provoking any other physiological changes known to him. The opportunity was then afforded to study the gaseous exchange, energy consumption, and ionic equilibria in the pancreas under conditions of rest and activity. Since the pancreatic juice is unlike most other body fluids in chemical composition and since the mode of stimulation is hormonal, we hoped to throw some light upon the mechanism of its secretion.

Since there are only a few published papers to which one may refer for comparisons of results or to supply lacking data, we shall omit a separate review of the literature.

The experiments represented in this paper, unless otherwise stated, were of one general plan. The rate of blood flow through the pancreas was measured. The arterial and venous pancreatic blood and pancreatic juice were collected and analyzed for various constituents. The results of these analyses and the interpretation of them furnish the basis of this paper.

METHODS. A. *Preparation of animals.* Dogs of 12 to 18 kgm. weight were used. They were fasted for 18 to 24 hours and anesthetized with barbital-Na, amytal-Na or chloralose. The abdomen was opened and the entire pancreas separated from the duodenum by a continuous row of small ligatures. Care was taken to injure neither the pancreatico-duodenal vein nor the branches from the pancreas to it. The inferior pancreatico-duodenal vein near the head of the pancreas was ligated. All branches draining into the pancreatico-duodenal vein which were not of pancreatic origin were ligated. The blood flow measured in the pancreatico-duodenal vein represented only pancreatic blood. A tight rubber ligature was placed

[1] This investigation has been made with the assistance grant no. 195 from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

around the pancreas just above where the pancreatico-duodenal vein leaves the pancreas. Thus we were able to measure or collect all of the blood and juice from the known portion of the gland. The femoral artery was cannulated for taking samples of blood. The saphenous vein was cannulated and connected to a constant injection apparatus. All accessory ducts were ligated. The pancreatic duct was cannulated for the collection of pancreatic juice. At this point the animal was carefully examined and all bleeders tied. Then 20 mgm. per kgm. of heparin were injected intravenously and the same amount injected per hour by means of the constant injection apparatus into the saphenous vein.

The inlet tube of the stromuhr was then quickly connected to the pancreatico-duodenal vein for measuring the rate of blood flow. In our experience this was completed within three minutes so that the gland was not exposed to asphyxial conditions for a significant period of time. Following the operative procedure, about an hour was allowed to elapse before the experiment was started in order that any asphyxial effects upon the pancreas would be dissipated.

B. *Measurement of blood flow.* Bennett and Still (1933) constructed the blood flow apparatus especially for this study. It possesses the following advantages: 1. The back pressure is so small (less than 6 mm. H_2O) that it may be used on the venous side of an organ, thereby leaving the vasomotor nerves on the arteries intact. 2. It is automatic and simple to operate and maintain. 3. It is very accurate. Figure 1 shows the construction details of the apparatus together with the device for drawing samples of blood.

This apparatus was constructed using certain of the principles found in the stromuhrs described by Pavlov (1887), Montgomery and Lipscomb (1929), and Hemingway (1931). It has been described in detail in this paper because we believe the improvements we have made enable one to measure the rate of the blood flow with greater accuracy and less mechanical trouble than has heretofore been possible.

The apparatus shown in figure 1 consists of three separate parts: 1, the

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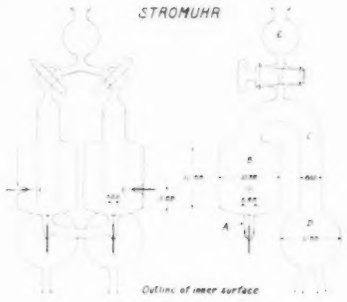
Fig. 1. The structural detail of the stromuhr, valve and operating circuits:

1, power relay; *2*, power relay; *3*, signal magnet; *4*, 3000 ohm rhoestat; *5*, polar relay (1200 ohm); *6*, T.P.D.T. switch; *7*, 800 ohm relay; *8*, 2000 ohm resistance; *9*, 15,000 ohm rheostat; *10*, stromuhr: *A*, chloroform chamber; *B*, copper sulphate chamber; *C*, oil chamber; *D*, blood chamber; *E*, filling chamber; *11*, valve bar; *12*, stromuhr outlet; *13*, stromuhr inlet; *14*, push solenoid; *15*, pull solenoid; *16*, blood sample tube: *F*, Ringer's reservoir; *G*, one way stopcock; *H*, one way stopcock; *I*, oil chamber; *J*, blood chamber; *K*, two way stopcock, $\frac{1}{4}$ inch bore; *L*, sample outlet; *M*, position of pinch clamp; *17*, front view of stromuhr and valve; *18*, side view of stromuhr and valve, showing valve construction and relationships of the tubes leading from the stromuhr to the animal.


STROMUHR
Outline of inner surface

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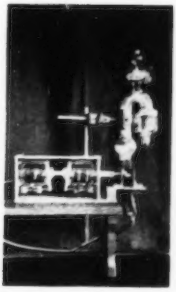

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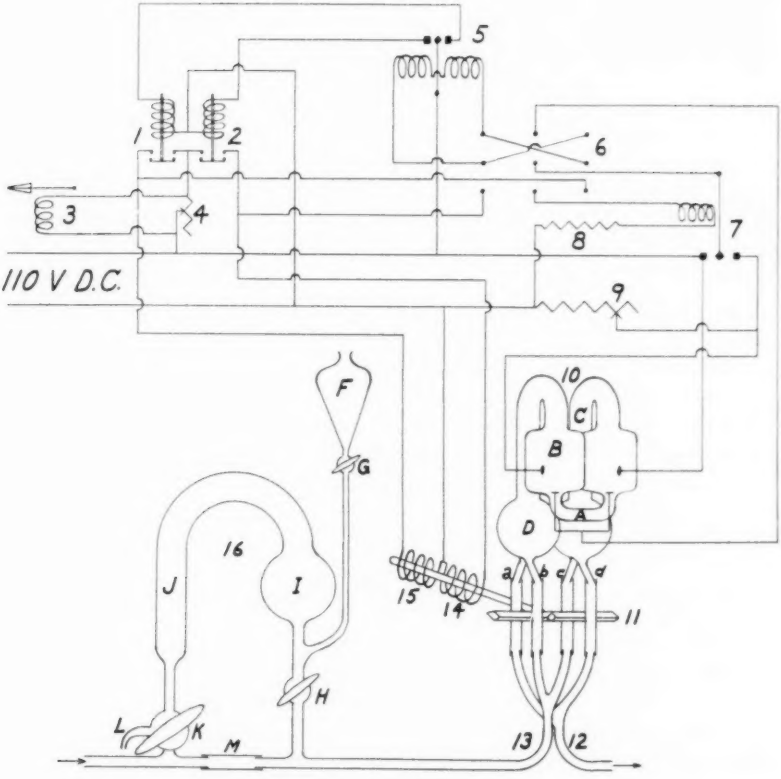
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Fig. 1

sampling device (16); 2, the stromuhr and operating valve, and 3, the relays, which operate the solenoids of the valve. The stromuhr is filled as follows: The cross arm *A* is filled with $CHCl_3$ level with the lower electrodes, then as much extra $CHCl_3$ is added as is desired per volume-shift. We found it desirable to adjust the volume-shift to $\frac{1}{3}$ or $\frac{1}{4}$ of the rate of flow per minute. Then each chamber *B* is $\frac{3}{4}$ filled with a saturated $CuSO_4$ solution saturated with $CHCl_3$. The balls *D* are half filled with heparinized saline solution. The remaining space in *B*, *C* and *D* is then filled with a low viscosity mineral oil. Balls *D*, rubber tubes from *a*, *b*, *c* and *d*, glass tubing *12* and *13* and rubber tubing leading to the venous cannulae are coated with melted vaseline.

The operation of the stromuhr: The diagram shows that the solenoids *14* and *15* are controlled through power relays *1* and *2* which in turn are operated by polar relay *5*. Polar relay *5* is operated by two sets of electrodes contained in the stromuhr chambers (*A* and *B*). If solenoid *14* (push) is functioning, the valve bar *11* will compress the rubber tubes leading from *b* and *d* so that the blood will enter the stromuhr through *c* and leave through *a*. The blood entering *D* will displace chloroform in *A* until one of the electrodes in *A* is exposed to the $CuSO_4$ solution. This activates the polar relay *5* and in turn opens solenoid *14* and closes solenoid *15*, so that the direction of flow of blood in the stromuhr is reversed. Switch *6* reverses the polarity of the electrodes to prevent deplating the electrodes and should be reversed every 4 to 6 hours. Relay *7* prevents gassing at the electrodes by disconnecting the current from the actuated side of the stromuhr and applying it to the side which will next be actuated. The figure shows structural details of the apparatus. This equipment has measured the blood flow in our experiments with great accuracy. Except in cases where the change in rate is enormous (over 300 per cent), the error has been under 0.5 per cent which, in a problem such as the one presented here, is not the largest error encountered.

Suggestions for filling stromuhr: 1. The stromuhr is clamped in an upside down position and the chloroform admitted through the bottom, the upper stopcocks being closed. This enables one to introduce a more accurate volume of chloroform into chamber *A*. Place the stromuhr in an upright position, rotating it in such a manner that all of the chloroform will drain into chamber *A*, then attach the rubber tubes to the balls *D*.

2. After connecting the stromuhr to the valve, by suction on *E*, draw melted wax into the balls *D* to leave a thin coating.

3. In a similar fashion, draw the copper solution up through tube *C* permitting enough to flow over into chamber *B* so that it is $\frac{3}{4}$ filled. Then allow the copper solution in tube *C* to be discharged.

4. Open both stopcocks and under pressure fill both chambers *C* with saline solution almost up to the bend. Then clamp tubes to one ball and

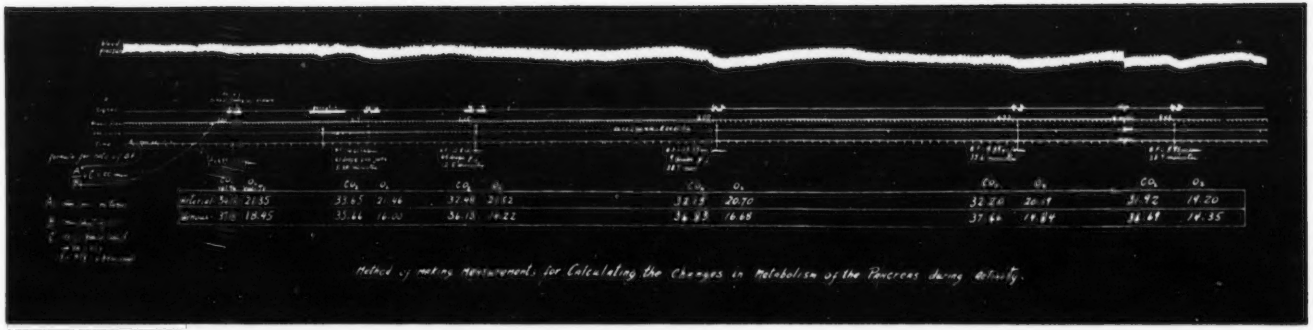

Fig. 2. A record taken from an experiment which illustrates the method of making measurements for calculating the changes in the metabolism of the pancreas.

close the stopcock on the same side. Fill *E* with oil and by permitting the saline to run out the oil will be drawn in. This is repeated until the entire chamber, with the exception of ½ of the balls and tubes connected to them, is filled with oil. The tubes and the lower half of the balls are filled with saline solution.

The sampling device is represented by (16). Stopcock has 6 mm. bore. With stopcocks *K* and *H* closed, the blood from the pancreas passes directly into the tubes leading to the stromuhr. To take sample: open stopcock *K* and *H* and place clamp on rubber tube at *M*. Blood enters *J* forcing oil into *I* and an equivalent volume of Ringer's solution flows through *H* and thence through the stromuhr. When sufficient blood has entered *J*, remove clamp at *M* and close stopcocks *K* and *H*. To remove blood sample: adjust stopcock *K* to connect *J* and *L;* dip *L* into a receiving tube under oil; open stopcock *G* which permits Ringer's solution to enter *I* and force oil and blood out through *L*. The blood is then under oil and may be manipulated in any way desirable. It has not been exposed to the atmosphere at any time.

Figure 2 shows a record taken from an experiment and illustrates the method of making calculations.

The method of calculating the rate of blood flow from the data was as follows: The expression $\frac{A}{B} \times C$ = cc. per minute when A = mm. per minute on the time record; B = mm. per shift on the blood flow record; C = cc. of blood per shift. The rate at the time of the samples A7 and V7 on figure 2 was calculated from these figures: 1 minute = 8.1 mm.; 1 shift of blood = 3.00 mm.; volume per shift = 4.89 cc. Thus the rate of flow at the time was 13.2 cc. of blood per minute.

C. The CO_2 and O_2 of the blood and pancreatic juice were determined by the method of Van Slyke and Neill (1924).

D. Water content of blood, juice or tissue was determined by heating to constant weight at 105°C.

E. Glucose was determined upon zinc filtrates by the method of Shaffer and Hartman (1921).

F. Lactic acid was determined using the procedure of Friedman and Graeser (1933).

G. Glycogen was determined by the procedure described by M. Sahyun (1931).

H. The secretin used in these experiments was from one sample prepared by one of us (E. U. S.) by a method similar to that described by him, and further purified by a method involving the use of picric acid, flavianic acid, and liquid ammonia which will be described in a separate paper.

I. Precautions in handling the samples of blood: cyanide, fluoride and the ice bath were used to minimize changes in the shed blood. Samples of

blood and sera were kept in an ice bath in tonometers over mercury pending analysis.

EXPERIMENTAL RESULTS. The gaseous exchange of the pancreas under conditions of rest and hormonal stimulation.

a. *Control experiments.* It was noted in some of our first experiments that the withdrawal of samples of blood caused a progressive fall in blood pressure and in the rate of blood flow through the pancreas. Although this occurred in only part of the experiments it was felt that control experiments should be carried out in which five or more samples each of arterial and venous blood were drawn without the injection of secretin. Three such control experiments were carried out. Figure 3 shows the results of one control experiment. The results indicate that unless the blood flow is diminished by more than 50 per cent there will be no important

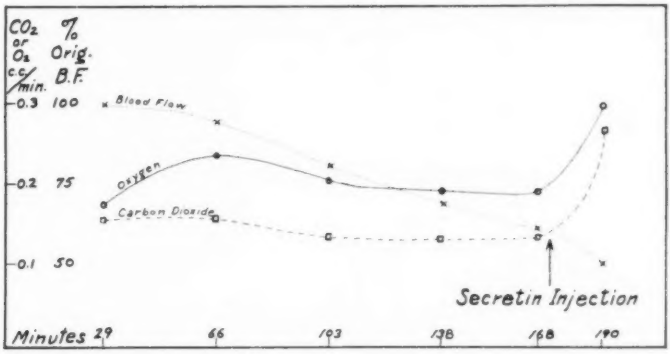

Fig. 3. Control experiment showing that a reduction in the rate of blood flow through the pancreas is without effect upon the rate of metabolism of the gland.

effect upon the rates of oxygen consumption and carbon dioxide output by the gland. As a matter of fact, we have several experiments in which the rate of blood flow through the pancreas has decreased as much as 50 per cent or more without materially influencing the rate of gaseous exchange of the gland. It would seem, therefore, that the rate of blood flow through the pancreas only bears a relation to the rate of metabolism in so far as asphyxia will develop from too slow circulation. This indicates that the pancreas is not nearly so susceptible to reduced oxygen tension as are some of the other glands of the body; e.g., submaxillary. In 2 or 3 of our experiments (data not included in this paper) the rate of blood flow diminished during the experiment to $\frac{1}{4}$ or $\frac{1}{5}$ of the control values. However, the rate of gaseous exchange was not materially affected. This was particularly interesting to us because in some cases when the blood was flowing very slowly the oxygen content of the venous blood was found to be as low as

⅕ of that of the arterial value. It should be stated, however, that none of the data reported in this paper have been drawn from experiments in which there have been such marked changes in the rate of blood flow.

b. *Effect of a single injection of secretin.* These experiments were carried out as in the control group except that after taking two control samples of blood a single injection of secretin was made intravenously. Samples of blood were taken during secretion and continued until the gland was again in the resting state, as indicated by the return of its metabolism to normal. Figure 4 shows the results of one typical experiment.

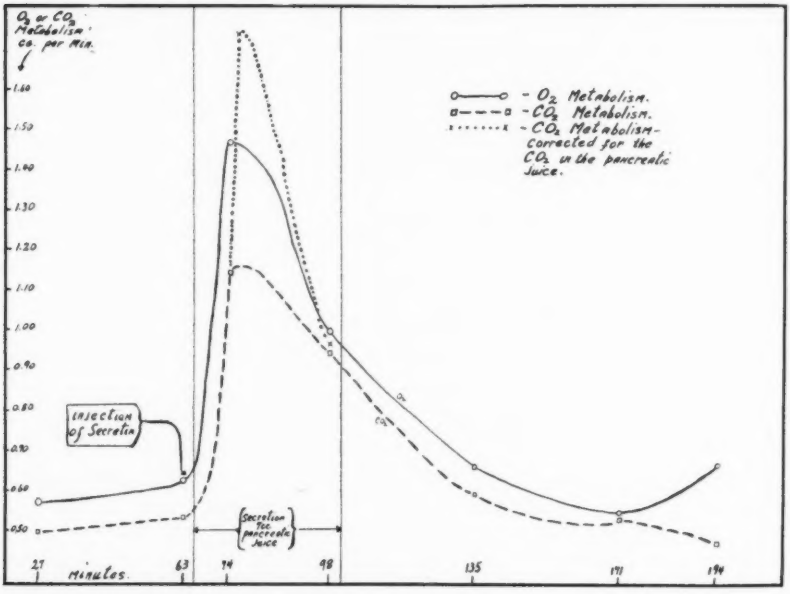

Fig. 4. The effect of a single injection of secretin on the gaseous metabolism of the pancreas.

The procedure was then modified to enable us to more clearly define the oxygen consumption and CO_2 production curves, by drawing more frequent samples of blood during the period of activity. It was observed that for a short time after the beginning of secretion, the venous CO_2 was diminished to values approaching those of the arterial blood. Table 1 shows the data and calculations from one such experiment. In this particular experiment, due to the extremely good condition of the animal, we were able to continue the work and complete two experiments. Although this experiment is similar to several others, it will be described in detail because of its completeness.

Figure 5 shows that very shortly after the injection of secretin the oxygen consumption began to rise and quickly reached a maximum. The oxygen debt was not paid until about 20 minutes after the secretion had stopped. The CO_2 production curve as calculated from the A-V difference at first was decreased. After about ten minutes, however, the CO_2 output increased greatly and did not return to the control value until some time after the secretion had stopped.

In the first part of this experiment 0.2 mgm. of secretin was injected. This led to the secretion of 2.5 cc. of pancreatic juice. The extra oxygen

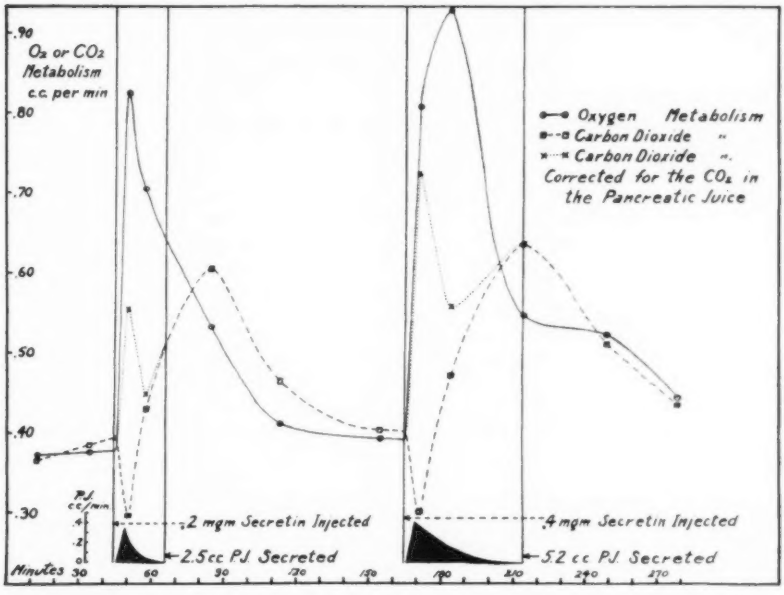

Fig. 5. The effect of a single injection of secretin on the gaseous metabolism of the pancreas.

used during the process was 10.49 cc. and the extra CO_2 produced amounted to 9.63 cc. as calculated from our data. In the second part of the experiment 0.4 mgm. of secretin was injected with the secretion of 5.2 cc. of pancreatic juice. The extra oxygen and CO_2 were 20.64 cc. and 18.04 cc., respectively. The calculated R.Q.'s for these two periods were 0.91 and 0.87.

A most interesting point seen in the experiments is the fall in A-V CO_2 difference during the first few minutes after stimulation. This was further investigated by several experiments so designed as to throw light upon the situation by drawing frequent samples of blood during the first

few minutes after the injection of secretin. In three of eight experiments the venous CO_2 actually fell to below the arterial CO_2. The data from one of these experiments is given in table 2. In one of the experiments it

TABLE 1

The data from an experiment which show the changes in the metabolism of the pancreas following the intravenous injection of secretin

PERIOD NUMBER	TIME OF PERIOD	TOTAL BLOOD FLOW	RATE OF BLOOD FLOW	ARTERIAL		VENOUS		RATE OF METABOLISM AT TIME OF SAMPLE		CORRECTION FOR CO_2 IN PANCREATIC JUICE	CORRECTED RATE OF METABOLISM AT TIME OF SAMPLE	
				CO_2	O_2	CO_2	O_2	CO_2	O_2		CO_2	O_2
	minutes	*cc.*	*cc./minute*	*cc./per cent*	*cc./per cent*	*cc./per cent*	*cc./per cent*	*cc./minute*	*cc./minute*	*cc./minute*	*cc./minute*	*cc./minute*
	0											
1	12.2	265	16.28	37.66	23.59	39.83	21.39	0.353	0.360		0.353	0.360
2	22.8	401	16.38	34.90	23.50	37.18	21.27	0.373	0.365		0.373	0.365
Injection of 0.2 mgm. secretin												
3	6.6	107	15.18	33.95	23.50	35.82	18.14	0.284	0.814	0.270	0.554	0.814
4	7.56	98	12.73	32.90	23.50	36.18	18.05	0.420	0.694	0.031	0.451	0.694
5	25.9	328	12.13	32.80	22.85	37.68	18.56	0.592	0.521		0.592	0.521
6	28.8	318	13.15	32.85	22.10	36.29	19.05	0.452	0.401		0.452	0.401
7	41.0	552	13.20	34.19	21.35	37.18	18.45	0.395	0.383		0.395	0.383
Injection of 0.4 mgm. secretin												
8	5.32	83	14.62	33.65	21.46	35.66	16.00	0.294	0.798	0.429	0.723	0.798
9	12.8	152	12.57	32.48	21.52	36.15	14.22	0.461	0.918	0.100	0.561	0.918
10	28.7	372	13.35	32.13	20.70	36.83	16.68	0.627	0.537		0.640	0.537
11	35.6	352	9.39	32.20	20.19	37.66	14.84	0.513	0.502		0.513	0.502
12	27.7	362	8.95	31.92	20.20	36.69	14.35	0.427	0.434		0.427	0.434

TABLE 2

The data from an experiment in which the CO_2 content of the venous blood fell below that of the arterial blood

TIME	ARTERIAL		VENOUS		CORRECTED FOR CO_2 IN JUICE	
	CO_2	O_2	CO_2	O_2	CO_2	O_2
	cc./per cent	*cc./per cent*	*cc./per cent*	*cc./per cent*	*cc./minute*	*cc./minute*
Control	16.65	20.65	22.90	12.51	0.150	0.200
3 minutes after secretin	15.61	20.18	13.04	10.14	0.580	0.439
5 minutes later	16.16	20.88	21.38	5.79	0.478	0.360
10 minutes later	16.03	18.15	23.03	6.55	0.233	0.258
1 hour later	16.33	17.69	23.11	8.04	0.144	0.205

almost equalled the arterial CO_2 and in the remaining four experiments it was only slightly higher than the corresponding arterial CO_2 values but lower than the resting venous values. This, of course, brings up the

fundamental question as to whether the bicarbonate of the juice is entirely metabolic in origin or whether some of it comes directly from the blood.

c. *The effect of continuous hormonal stimulation.* These experiments were of the same general character and plan as those cited under *b* except secretin was continuously injected over a period of about one hour. In this way the pancreas was maintained in a state of great activity for a relatively long period of time. It was hoped to determine if prolonged activity resulted in changes other than those already noted. Figure 6 shows graphically one of our experiments.

It will be noted that the changes in respiration resemble those due to the single injection of secretin except that they last longer. It is interesting to note that the average rate of oxygen consumption per gram of wet tissue in our studies was: Resting 0.029 cc. per gram per minute and during

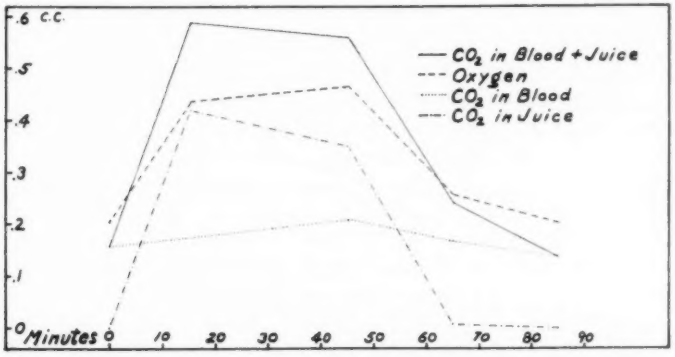

Fig. 6. The effect of continuously injecting secretin on the gaseous metabolism of the pancreas.

activity 0.062 cc. per gram per minute. Gerard and Still (1932) using the Warburg technique on excised rat's pancreas found the oxygen consumption to be 0.01 cc. per gram per minute resting and 0.014 cc. per gram per minute after the addition of secretin.

Table 3 gives the summary of eleven experiments selected because of the fact that they were completed without mishaps of any kind which would cause us to question the results. The extra R.Q. of work from these data suggests that the energy for the work of secretion comes from carbohydrate. Therefore, we planned experiments to investigate that point.

d. *Study of carbohydrate utilization by the pancreas.* The general plan of these experiments was the same as heretofore described. Larger samples of blood were taken to allow for duplicate analyses of CO_2, O_2, glucose and lactic acid. Figure 7 gives one typical experiment.

In none of our three experiments have we been able to show a quanti-

tatively consistent change in the glucose used by the gland during activity as compared with the resting state. In all of our experiments the A-V difference of glucose per minute decreased with the onset of activity. Cer-

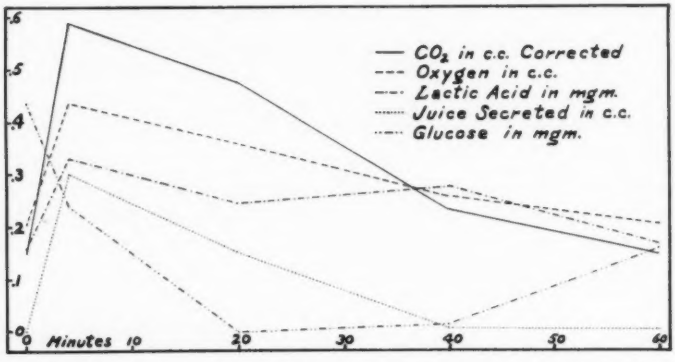

Fig. 7. The effect of secretin upon the respiration, glucose and lactic acid metabolism by the pancreas.

TABLE 3

The data from 11 experiments, showing the resting metabolism of the pancreas, and the respiratory quotient

NUMBER	RESTING CO_2 (DRY WEIGHT)		RESTING O_2 (DRY WEIGHT)		RESTING R.Q.	EXTRA R.Q. OF WORK
	cc./minute	*cc./gram/minute*	*cc./minute*	*cc./gram/minute*		
1	0.134	0.061	0.196	0.089	0.69	0.96
2	0.321	0.066	0.354	0.073	0.91	1.04
3	0.365	0.100	0.507	0.139	0.72	1.22
4	0.363	0.100	0.395	0.109	0.92	0.98
5	0.543	0.180	0.629	0.212	0.86	0.94
6	0.341	0.095	0.463	0.129	0.735	1.04
7	0 224	0.042	0.310	0.057	0.72	0.97
8	0.338	0.131	0.407	0.158	0.83	0.99
9	0.373	0.102	0.365	0.100	1.02*	0.95
10	0.395	0.109	0.383	0.105	1.03*	0.88
11	0.151	0.108	0.201	0.143	0.751	1.60
av	0.3226	0.0994	0.3827	0.1194	0.792	1.05
s.d.	±0.0351	±0.0111	±0.0380	±0.0130	±0.029	±0.061

* Not used in calculating ad.

tainly this is not evidence for a decreased utilization of glucose. Rather we believe it to represent glucose added to the venous blood by the active gland. This would diminish the A-V difference. In all of the experiments the lactic acid produced was increased as is shown in figure 7.

These findings together with the calculated R.Q. of activity (see table 3) caused us to look farther for the source of the increase in carbohydrate metabolism by the gland during activity. It seemed possible that a glycogen breakdown furnished carbohydrate for oxidation and added glucose and lactic acid to the venous blood. Four experiments were carried out to test the hypothesis: samples of the pancreas were removed before, during and after secretion. They were analyzed for glycogen. We have found as great differences in the glycogen content of adjacent parts of the resting pancreas as we have during and after activity. It seems, therefore, that the pancreas is not of uniform composition.

Discussion. Probably the most interesting finding in these experiments was the diminution of the A-V difference in the CO_2 during the first stage of secretion. A consideration of the data from experiments similar to the one cited in tables 1 and 2 may elucidate somewhat the question as to the source of HCO_3 in the juice, which is a somewhat moot question. Woiner (1928) believes the CO_2 of the juice is dependent upon (and therefore arises from) the blood serum. He found that continuous secretion by the pancreas led to a lowered alkali reserve due to loss of fixed base. Hammarsten and Jorpes (1928) did not confirm Woiner. They found that continuous secretion did not lead to a diminution of the alkali reserve. They found acid to accumulate in the gland during activity. They hold that the base comes from the blood and the CO_2 from the gland. Ball (1930) from an excellent series of experiments came to the same conclusion, "since the juice bicarbonate is remarkably independent of the serum bicarbonate." The views of these workers are either partly or totally in disagreement. We might point out that, while their experiments were excellently planned and executed for the study of the total metabolism of pancreatic secretion, they were unable to express their results quantitatively with respect to time and therefore failed to observe the first phase of secretion. Our attempt to express our data quantitatively with time and to measure the rate of blood flow brought these considerations to our attention.

Since in every experiment planned to detect it, we observed the diminished A-V difference in CO_2 during the first few minutes after the injection of secretin, we are convinced as to the correctness of our observations (see table 1 and fig. 5). Probably our data entitle us to consider further the mechanism involved and the source of HCO_3 in the pancreatic juice.

Our data suggest in the resting gland there is little passage of substances other than oxygen from the blood into the gland with the exception of that which goes into the formation of lymph (which has not been directly studied). On the other hand CO_2 at least passes from the gland into the blood. With the onset of secretion the venous CO_2 becomes diminished and HCO_3 appears in the juice. After a few minutes, although the CO_2 of the juice remains high or even increases in concentration, the venous

CO_2 rises above the resting concentration. This, of course, may be interpreted as indicating that the entire CO_2 of the juice is metabolic in origin, and that more CO_2 is produced by the gland, at a given time, than is secreted in the juice, because of the raised level of the venous CO_2 during the second phase of the secretion. However, we cannot overlook the three experiments in which, during the early phase of secretion, we found the venous CO_2 lower than the arterial CO_2 (see table 2). These results cause us to lean toward the following explanation: During the first phase of secretion the juice CO_2 arises partly from the blood and partly from the metabolism of the gland. This seems to be particularly so if the pancreas has received a maximal secretin stimulation. After a continuous "secretary state" has been established the increased metabolic CO_2 of the gland is more than enough to supply the juice and, therefore, a part of it passes into the blood. Our evidence is therefore in agreement with and has contributed to the theories advanced by Hammarsten and Jorpes and Ball.

We have carried out equilibrium experiments to determine directly the change in fixed acid and base of the arterial and venous blood, before secretion, at the height of secretion, and after secretion. These experiments will be presented in another paper.

SUMMARY

A method has been described for the study of the metabolism of a gland in situ. The method has been applied to the pancreas with results which we feel enable us to draw the following conclusions.

1. The secretion of pancreatic juice is accompanied by an increased consumption of oxygen. A considerable oxygen debt usually develops and is usually "paid" within 30 minutes after the gland has ceased to secrete.

2. At the beginning of the secretion of juice, there is a fall in the pancreatic venous CO_2 due to the removal of much of the metabolic CO_2 of the gland by the juice. In some cases the venous CO_2 may fall below the value obtained for the arterial blood. Soon, however, the metabolic CO_2 is sufficient to supply the $BHCO_3$ for the juice and to increase the venous CO_2 above the resting level. We, therefore, believe the principal source of CO_2 in the juice is from the metabolism of the gland. As indicated a small portion may arise during the beginning of secretion from the blood.

3. The average resting R.Q. of the gland was found to be 0.79. We believe that figure is probably too high because it is difficult to study the gland under conditions of absolute rest. The average R.Q. of work (extra R.Q.) was found to be 1.05.

4. Whether or not carbohydrate is the source of energy during activity in the pancreas is an open question.

It is a pleasure to express our deep appreciation to Dr. T. E. Friedman, who has been of inestimable assistance to us in applying his method for the determination of lactic acid to the problem.

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A COMPARISON OF THE CHARACTERISTICS OF AXONS THROUGH THEIR INDIVIDUAL ELECTRICAL RESPONSES[1,2]

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Studies with the cathode ray oscillograph have shown that the fibers composing a peripheral nerve range widely in their reactivities. For instance, a large nerve, such as the sciatic, contains fibers conducting at rates varying as widely as 50 to 1 with an even wider irritability range as determined by induction shocks. But neither in this nor in other nerves is there an even numerical distribution of fibers with respect to these properties. The uneven numerical distribution of fibers with respect to conduction rate is manifested in the consistent elevations which appear on the maximal, conducted action potentials of large nerves.

Attempts have been made to trace to their sources the fibers contributing their potentials to the several elevations and to ascertain the functions the component fibers subserve (Erlanger, Bishop and Gasser, 1926; Erlanger, 1927; Erlanger and Gasser, 1930; Bishop and Heinbecker, 1930). Bishop and Heinbecker recognize four types of fibers, namely A, B_1, B_2 and C, present in varying combinations according to nerve and species. "The ordinary mixed somatic nerve," they say in one of their latest publications (Heinbecker, Bishop and O'Leary, 1932), "gives rise to all four. The fibers responsible for the A component," they go on to say, "are the large thickly myelinated ones known to include, primarily, somatic motor and sensory elements. The B_1 component includes primarily the somewhat smaller and frequently somewhat more thinly myelinated fibers known to be visceral afferents. These two potential components have characteristic physiological properties which permit their differentiation from the B_2 and C components possessing properties of a much slower order. The fibers responsible for the B_2 potential are the smallest thinly myelinated ones found in the nerve. Unmyelinated fibers give rise to the

[1] The experimental data in this paper are taken in part from a dissertation presented by Edgar A. Blair in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Washington University, submitted 1933.

[2] This work was made possible in part by assistance from a grant made by the Rockefeller Foundation to Washington University for research in science.

C potential." These four types, they say (Bishop, Heinbecker, and O'Leary, 1932), "can be differentiated by their action potentials."

Measurements of the reactivities of the component fibers of a nerve have been based upon the superimposed responses of great numbers of fibers contributing to each of the several distinguishable features of the composite potential. Such a method is very apt to overemphasize the properties of the fibers contributing to the features used as guides and consequently to miss any transitional reactivities there may be between them. In order to obviate this danger we have sought to determine the reactivity range by methods that would search out individual fibers of a nerve. The results of this method of investigation have been to show that all of the reactivities of fibers, not conductivity only, but also irritability, however measured (excepting by chronaxies), absolute refractoriness, and time to maximum and amplitude of their action potentials are in continuous range from the most to the least reactive of the fibers, and so also are the summation intervals, excepting in fibers conducting more slowly than 2 m.p.s.

General methods. A recording mechanism for the study of the action potentials of single fibers must *a*, be able to reproduce with no significant distortion input potentials with an effective frequency of 5000 per second; must *b*, be sufficiently sensitive to permit the reading of these potentials to 5μv, and *c*, yield, by a simple technique, records of single events which may be read significantly to 0.01σ. The cathode ray oscillograph plant as originally designed (Gasser and Erlanger, 1922) fulfilled the first requirement. The second requirement has been met by the addition of a fifth stage to the original amplifier (see fig. 1) thus increasing the voltage amplification up to 2,200,000. With low input resistance ($50{,}000\omega$) and maximum amplification, input potentials can be read significantly to 1μv. If speed were sacrificed by inserting parallel capacity, as Rijlant (1932) has done, still higher sensitivity could easily be attained. The third requirement has been met by replacing the Johnson cathode ray oscillograph with that of von Ardenne. With 2000 volts anode potential, the luminosity of the latter is such that photographs can be made of the spot traveling at the required speed. However, the spot can be focussed so sharply that satisfactory contact prints of high speed, single deflections can be made on relatively insensitive ("Process") film without exercising any particular precautions such as had to be employed previously (Gasser, 1928), and this simpler technique has usually been employed.

The spot is now moved across the X, or time axis, by the logarithmic fall of potential across a condenser, *SC*, discharging through a variable resistance, *SR*, the value of the latter determining the rate of the deflection. A brief closure of the series key, *S*, very rapidly recharges the condenser from a potential source, *S*, supplied by rectified alternating current. Amplification can be varied through a voltage divider connected with the grid

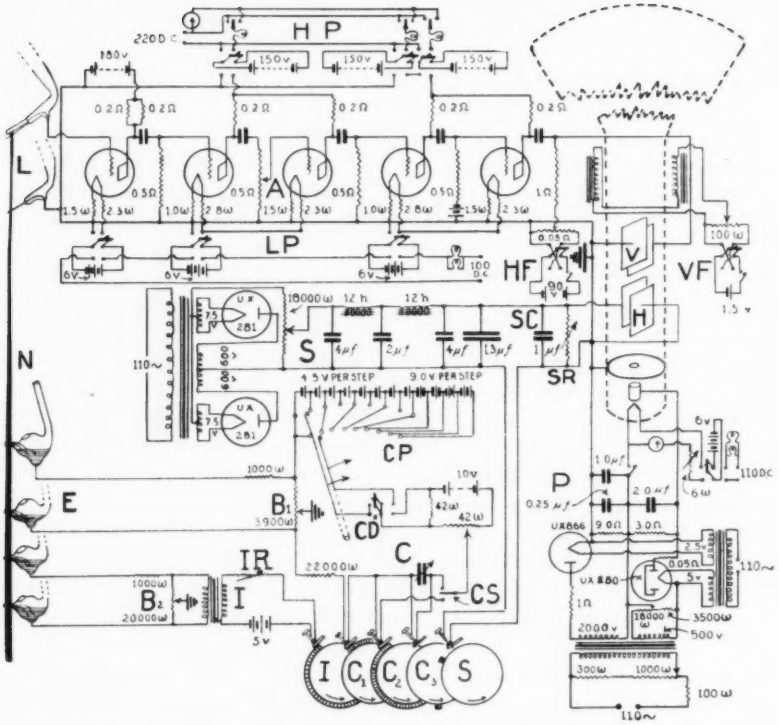


Fig. 1. Stimulating, amplifying and Braun tube circuits.

A. Amplifier employing 5 Western Electric 102-D tubes. *HP*. High potential amplifier plate batteries. First stage heavy duty "B" batteries. Other stages Edison cells. *LP*. Low potential acid filament batteries. *V*. Braun tube plates deflecting the spot vertically, recording input potentials (Y axis). *H*. Braun tube plates deflecting the spot horizontally, recording time (X axis). *VF*. Vertical electromagnetic field controlling resting position of spot on X axis. *HF*. Horizontal electrostatic field from voltage divider in parallel with the amplifier output, controlling resting position of spot on Y axis. *S*. Sweep circuit supplying potential for deflection of spot along X axis. *SC*. Precision condenser in timing circuit. *SR*. Precision resistance in timing circuit. Key *S*. Key initiating the sweep of the spot along X axis. *P*. A.C. operated power supply for Braun tube. *C*. Calibrated variable condenser for the condenser-constant current stimulating circuit. *CP*. Point switch controlling stimulating potential. *CD*. Voltage doubling switch for determination of chronaxies. Key C_1. Series make key initiating stimulus from condenser-constant current circuit. Key C_2. Series break key for breaking constant current circuit. Key C_3. Condenser discharging key, closing while C_1 is open. C_s. Selector switch; in up position circuit delivers condenser charges, in down position, constant currents whose durations are determined by the separation of C_1 and C_2. *I*. Porter inductorium. *IR*. Precision resistance controlling primary current. Key *I*. Break key in primary current. B_1B_2. Balancing circuits to reduce shock artifacts. *N*. Nerve. *E*. Low resistance nonpolarizable calomel stimulating electrodes. *L*. Lead electrodes.

of the third tube and was determined by calibrations carried out under the conditions of the experiment.

With maximum amplification it is possible to obtain a record of the action potential of a single axon by leading from a nerve as large even as the green frog peroneal, provided it contains an axon of outstanding irritability. The invariable, all-or-none nature of the response characterizes it as an axon potential. For the purposes of this research, however, it was essential to be able to identify in a single preparation the responses of each of a series of axons with reactivities illustrative of the entire range.

In the main, two preparations have been employed for this purpose. Use has been made chiefly of the entire length of the sciatic nerve of the green frog (*Rana pipiens*) from the spinal column to the tip of the third digit. This we designate the plantaris preparation. It was stimulated near its central end (with cathode proximal to lead) through calomel half-cells of a type differing slightly (see fig. 1) from those of Bishop, and the responses were led from its distal end through silver electrodes into the amplifier. The grid (distal) electrode is a minute spring clip designed to hold the end of the nerve (fig. 1). From frogs of medium size conduction distances up to 135 mm. are obtainable. The end from which the lead is taken measures only 20 to 50μ in diameter and contains 20 to 100 fibers with diameters ranging from some 12μ down to that of the smallest unmyelinated fibers. Presumably they are representative of all functional types contained in the sciatic, excepting possibly voluntary motor. The distal end of the nerve between the leads is probably not wholly free of branches, but the small branches produce no significant distortion of the action potential except that the potentials of fibers leaving the nerve here are consistently diphasic and cannot be made monophasic by killing the stretch distal to the point of egress.

Rarely have we been able to obtain more than twenty axon potentials from such a preparation. The discrepancy between the number of axons present and the number responding is attributed to peripheral anastomoses and to damage of some fibers in preparation. But whatever the cause of the discrepancy between the number of fibers present and the number responding to distant stimulation, it was fortunate that the number of active fibers was small for only then is it possible to identify the axon potential of each of the active fibers through irritability, conduction rate and configuration of axon potentials and then only when the latter happen to be sufficiently and advantageously spaced. The longer the preparation the more satisfactory is the spacing. Care was exercised to avoid injuring mechanically, and drying of the slender peripheral end of the nerve during its preparation. However we are not sanguine enough to believe that all of the fibers of a preparation were undamaged.

It is an easy matter to find in frogs skin nerves that contain fewer fibers

even than plantaris. But in the former all of the fibers at the lead respond to stimulation centrally and, since the distance of conduction is short, identification and measurement of the responses of the individual axons are much more difficult than with plantaris. The skin nerves, however, have the advantage that they lie free in the lymph sacs and, therefore, can be prepared without any other damage than that involved in cutting the two ends. They have been used mainly for the purpose of controlling in a minimally injured nerve the conclusions derived from the intensive study of plantaris.

Extra precautions must be taken after mounting these very slender nerves to prevent drying and also to prevent condensation of water on them. To this end the attenuated parts are mounted vertically in the moist chamber and drops of Ringer's solution, brought to the temperature of the nerve by means of a coil within the chamber, are permitted to flow over it, rapidly while the chamber is open and infrequently during observations. The nerve chamber is kept at a constant temperature. In many experiments in order to increase the accuracy of time measurements it has been held, by means of built-in refrigerating coils, to a temperature considerably below that of the room.

The keys operated by the metal segment wheels of the rotator consist of a single, nonconducting, short, stiff spring which carries one contact point. When properly adjusted with a clearance of about 0.1 mm. between contact points there is no demonstrable time variation in the case of the break;

Fig. 2. *1.* A contact print of 40 successive half maximal α spikes from the unbranched sciatic, showing the superimposability of the shock escape and of the conducted potential wave. The time is slower than in records *2* to *9* and the amplification less.

2–8. Serial contact prints obtained under exactly comparable conditions showing the variations in the response of the sciatic to a shock, uniform as above, but just threshold; records mounted so that the time from the shock is the same for all.

9. Deflection without stimulation, showing the noise level in *2–8.*

Speed of spot not accurately determined, but the time from the start of the first wave in *2* to its crest is considerably less than 0.2 σ. 10μv = 7 mm. 1/21/32. 23.3°C.

Fig. 3. Three cleanly separated spikes of axons of slow conduction; from plantaris. All subsequent records are from this nerve unless otherwise specified. In this and in subsequent figures numerals in front of waves give conduction rates in m.p.s.; under the waves, crest times in σ; over the waves, amplitudes in μv; and on the base line, time in σ. Where zero time is not noted in the figure it is at the left edge of the records. The slower the propagation the lower the amplitude. The lower record shows the change resulting from the entrance of a fourth axon spike (diphasic) between the first and second, as a result of a spontaneous increase in its irritability. 12/16/32. 17.4°C.

Fig. 4. Three successive responses of an axon stimulated at the lead by three successive identical threshold constant currents each lasting through the period of the response it produces, to show spontaneous variations in utilization period (and latency ?).

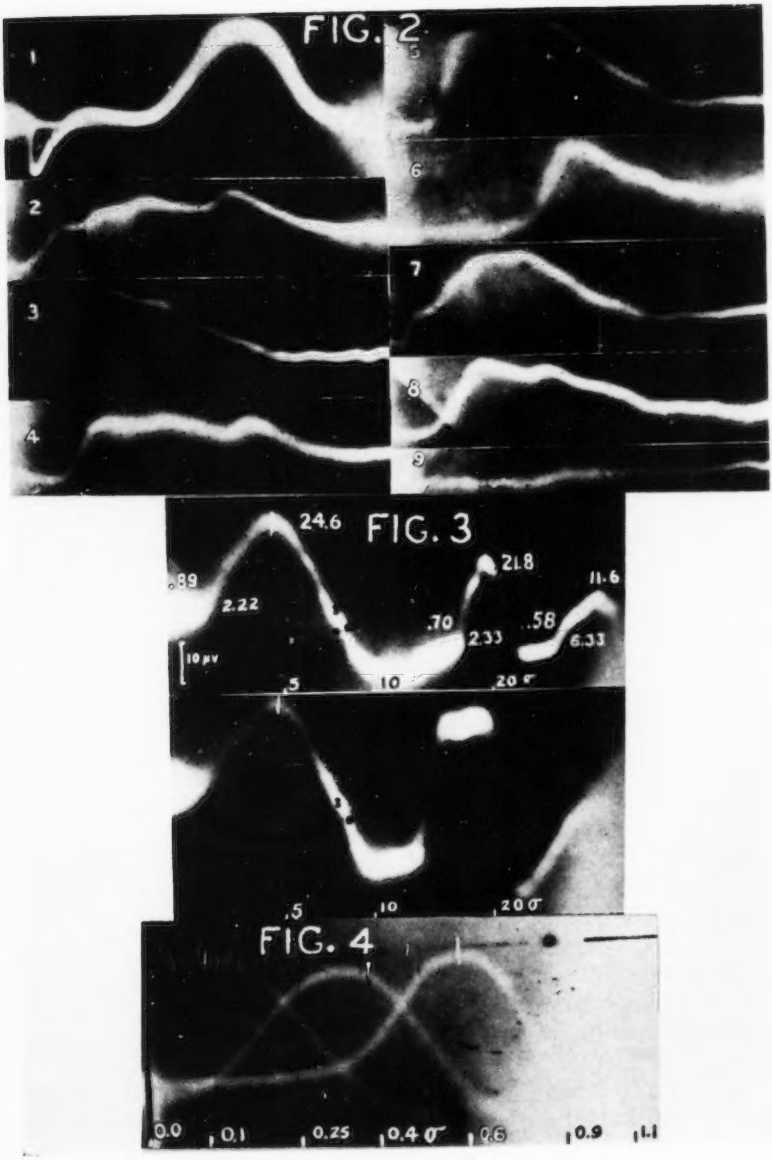


FIG. 2
1
2
3
4
5
6
7
8
9
24.6
FIG. 3
.89
2.22
.70
2.33
21.8
.58
6.33
11.6
10 μv
.5
10
20 σ
.5
10
20 σ
FIG. 4
0.0
0.1
0.25
0.4 σ
0.6
0.9
1.1

Figs. 2–4

and the variation in the time of closure on the make is not over 0.01σ. Throughout observations, unless otherwise stated, the nerves have been stimulated steadily 58 times a minute.

RESULTS. *Momentary variations in axon state.* When we first began to use amplification high enough to render visible the responses of single axons (Blair and Erlanger, 1932; Blair and Erlanger, 1933a) we were struck by the kaleidoscopic appearance of threshold responses obtained from large nerves under absolutely constant conditions (see fig. 2). The variability of this picture, which apparently has been seen also by Monnier (1932), we now know is referable to spontaneous and independent variations of the reactivities of the responding axons, and before comparing axons with one another it will be necessary to consider those phases of variability that qualify comparison.

Variations in irritability. When a preparation containing a fiber of outstanding irritability is stimulated with shocks increased in strength by small steps from below the fiber's threshold, there are at first only rare responses. To elicit a spike with every shock it usually is necessary to increase the strength further by about 2 per cent. This increase in strength necessary to stabilize the response is far in excess of uncontrolled variations in shock intensity. Directly observed upon the screen of the Braun tube the shocks with any given setting do not vary perceptibly in height, and a variation of 1 per cent would be appreciable. Neither is there any appreciable variation in the configuration of the shocks. However, a variation of, say, 5 per cent in the duration of a shock lasting 0.03σ would scarcely be demonstrable, yet might alter considerably its stimulation value. That the shock is steady is proved by the fact that in large nerves a submaximal alpha spike, whose height changes demonstrably with a change in primary current of 0.25 per cent, shows no appreciable change with any given setting of the same stimulating circuit (fig. 2, *1*).

That this shifting irritability of the axon is spontaneous is readily demonstrable. One not infrequently obtains preparations containing two fibers equally irritable but with different conduction rates. When these two fibers are stimulated with threshold shocks all possible response variations are seen,—both fibers may respond to one shock, one only to the next, the other only to a third, or neither may respond. Variation in shock strength could produce no such variations as these; they must be due to spontaneous and independent fluctuations in the irritabilities of the two fibers.

Latency and its variations. The time intervening between successive threshold shocks and the resulting conducted axon spikes, or the shock-spike time, under exactly comparable conditions is not constant. The range of fluctuation in the case of the more irritable fibers may be as great as 0.5σ, but usually is about 0.2 to 0.3σ; in the case of the less irritable fibers

it may be more than 2.4σ (conduction rate 5.28, 24°C.). The extreme ranges for the different fibers could be determined by statistical methods only and this has not been done systematically. That these fluctuations are independent of variations in shock strength and are, indeed, spontaneous on the part of the fibers, can readily be demonstrated in preparations in which there happen to be two fibers with identical irritabilities and conduction rates but whose spikes are identifiable through differences in amplitude or configuration (see fig. 14). When such a preparation is stimulated with threshold shocks not only will the resulting picture exhibit the play referable to fluctuations in irritability, but, in addition, the shock-spike intervals will vary independently, first one spike appearing earlier, then the other, etc.

The extent of the play in position is not affected by varying the distance of conduction; indeed, it is seen, apparently undiminished in extent, when the axon potential is led directly from the stimulated locus. The play, therefore, develops at the stimulated point. This conclusion is confirmed by the fact that the shock-spike time can be stabilized by increasing the strength of stimulation. As the shock strength increases the play at first diminishes rapidly in extent and shifts forward; eventually it disappears but the shock-spike time continues to diminish indefinitely, though very slowly. Some of the forward shift, particularly when the shock becomes strong, is to be attributed to spread of stimulation, but how much it is impossible to say.

A better conception of what is happening under these circumstances is gained by recording the axon spike at the stimulated point. With the very high amplification necessary, the escape of the shock covers the start of the action potential. The axon potential crest, however, is clear and shock-crest time can be accurately determined. When the induction shocks are at threshold the crest position plays through more than 0.25σ (29.1°C.). With shock strengths that eliminate the play and, probably the greater part of the forward shift the shock-crest time is 0.45σ. By a method to be described later one can ascertain at the stimulated locus the crest time of the axon potential. In the present instance it measured 0.25σ. There is no assurance that in this experiment the maximum forward shift with increasing strength was attained. Furthermore, though the stimulating cathode and ground lead are one, the point stimulated and the point led from presumably are not the same. The former must be on the anodal side, the latter on the grid side of the electrode, a separation of 1 mm. certainly, possibly 2 mm. Assuming that the conduction rate was 20 m.p.s. 0.05 to 0.1σ would, therefore, have to be allowed for conduction time. It thus appears that with shocks considerably above threshold strength the shock-spike time probably is as long as 0.1σ and exceeds considerably the measurable duration of the shock (in this case 0.03 to 0.04σ).

Since a utilization period cannot by definition exceed the duration of the applied potential it follows that in this case the response to the strongest shock used was preceded by a latent period of the order of 0.06σ and the response to the weakest shock by a latent period of the order of 0.3σ. The play in shock-spike time in a nerve stimulated at threshold is due almost entirely to variation in latency indicative of a local play in reactivity.

Previous determinations of shock-spike time at the site of stimulation, 0.06σ by Erlanger and Gasser (1924) and 0.06 to 0.1σ by Bishop (1927), led to the conclusion that this period is little if any longer than the "effective duration" of the shock. Comparable values, it has just been seen, are obtained from single axons when the shocks used are as much above threshold as were those probably used to produce submaximal potentials in the earlier work. The direct demonstration of a latency of the response to a shock confirms the indirect demonstration made some years ago (Erlanger and Blair, 1931b).

Variations in utilization period. With constant current stimulation the fluctuations in the reactivity of the axons becomes more striking even than with induction shock stimulation. At the threshold the make-spike time play is much wider than that of the shock-spike time. This is the case not only when conduction is involved but also where the cathode of the constant current and the lead into the oscillograph are one (fig. 4). In an experiment of the latter character in which the shock-spike play at threshold was at least 0.25σ, the maximum recorded make-spike play at threshold was at least 0.5σ. In neither case was the recorded shift the maximum seen on the screen.

The remote possibility that the fluctuation in the shock-spike and make-spike times is due to shifting of the point stimulated along the nerve is rendered untenable by the fact that the fluctuation may be greater than the calculated time for conduction along the distance subtended by anode and cathode. Furthermore, the extent of the play is independent of the interelectrode distance in the range studied (0.2 to 2.0 cm.).

The fact that the shock-spike time in an axon, in observations involving conduction, becomes fairly constant when the shock strength is well above threshold does not definitely eliminate the possibility of spontaneous fluctuations in conduction rate. It does signify, however, that if such fluctuations do occur they are local, and that the conduction time as measured remains constant because such local differences balance.

There are many reasons for believing that this play in the state of the fibers is normal and may be independent of the treatment to which the nerve is subjected. Among others may be mentioned: *a.* The play is seen in all parts of the plantaris preparation including the thick unbranched portion. Here size of nerve and thickness of sheath rule out the possibility of accidental damage in preparation. *b.* The easily prepared unbranched,

dorsal skin nerve yields a similarly variable picture; *c*, and so do refrigerated preparations 24 to 36 hours old in which the demarcation potential must be insignificant.

The base line,—conduction rate. In this paper a number of activities of individual axons, distinguished by their action potentials, are to be compared. Since these activities cannot, in the present stage of our knowledge, be assigned to the anatomical units which they characterize, it becomes necessary to correlate the activities by measuring them in terms of some one activity. Conduction rate is the one that has been selected to serve as the common denominator.

Conduction alone of all of the activities that have been studied is at one and the same time a physiological response to a physiological stimulus (except at the locus of stimulation), occurs in regions removed almost entirely from the influence of abnormal contacts, and is not, so far as is known, affected by anatomical relations outside the axon itself. It is, furthermore, the most constant from moment to moment, and from point to point on the nerve, of the activities we have investigated. This constancy presumably is referable to the fact that, whereas all other activities are measured at a point, conduction time is the sum of the conduction times in the parts of the fiber, and even if conduction rate varied at random from part to part the total time over a considerable stretch might still remain constant from trial to trial.

Of other possible gauges, the relative irritabilities of the fibers of a nerve to some extent must be influenced by local anatomical relations of the responding fibers and, in different nerves, not only by that but also by differences in nerve diameter. Summation intervals are not readily measurable and have as yet an uncertain significance. As to refractory periods, those of the more irritable fibers can be measured easily and sharply, not, however, as will be seen, those of fibers of low irritability. The amplitude of the axon potentials in different nerves is affected by the amount of inactive shunting tissue; and time to maximum, for reasons to be given later, is apt to be a difficult measure to apply.

We compare with conduction rate certain manifestations of activity, such as irritability, refractory period and summation interval, measured at the proximal end of the nerve, and certain manifestations, such as amplitude and time to maximum of the axon potential, at the distal end. If such a comparison is to be valid, either all parts of the stretch must have the same mean reactivity, or, if they have not, the departure from the mean must follow some rule. Thus if conduction rate falls off toward the periphery, as Gerard and Marshall believe (1933), and if such slowing developed regularly and uniformly comparisons still would be valid. There are reasons for believing, however, that there may occasionally be discontinuous alterations in reactivity along an axon. There is, for example,

indubitable evidence of occasional axon branching in the sciatic-plantaris preparation, and this may be used to illustrate one of the possible sources of error when conduction rate is used as the common denominator. Figure 20, *A* shows three axon spikes initiated, it can be shown, by stimulation of a single axon which branches a considerable distance from the lead. That the three spikes come from a common trunk is proved by the unchangeableness of the picture under conditions which unquestionably would alter it were the potentials from fibers discrete throughout their length. Thus in the play that occurs at threshold stimulation either the entire triple deflection appears, or none at all; and the spacing between the spikes always is the same whether stimulation be by induction shock or by constant current. The spikes are not repetitive discharges in one axon. For 1, we have never seen repetition in an axon from stimulation with a threshold induction shock; 2, the picture is not that of repetition, the potentials of repeating spikes never falling to so low a relative height as does the third of these three; and 3, in a fiber repeating under the influence of a constant current the axon spikes would be spaced by at least 3σ whereas here the spacing is roughly 1σ.

The complications that branching might give rise to in a comparison of axon activities are many. Only one need be instanced. If, say, the two most rapidly conducting of these three daughter axons had left the preparations proximally to the lead, conduction time (consumed mainly in the branch, the smallest one) would have been unaccountably long in comparison with irritability (measured in the parent trunk). In view of such possibilities the wide discrepancies occasionally encountered in the usual relation of irritability to conduction rate cannot be regarded as significant.

As in previous publications from this laboratory conduction time is taken to be the shock-spike interval. This interval may be quickly determined by making the shock artifact coincide with the initiation of the sweep along the time line and adjusting the charging resistance of the condenser in the sweep circuit so that the spike appears when the sweep has been 63.2 per cent completed. This point, which is marked by a dot on the face of the tube, has been chosen because, with a $1\mu f$ condenser, the transit time of the spot to the dot is 1σ for each 1000ω in the charging circuit. The charging resistance may be read to 20ω or to 0.02σ. Since the total conduction time for the quickest fiber in a full length plantaris preparation is of the order of 7σ this represents a minimum accuracy of 0.3 per cent in the measurement of this time.

In order to minimize in these measurements the disturbing influence of latency, shock strength is always increased to the point where the play due to latency variations ceases. In the case of the fastest fibers the interval between shock start and initiation of the impulse locally may be taken as 0.1σ. As no allowance is made for this in our determinations the conduc-

tion rates for the fastest fiber in long plantaris preparations are too slow by something over 1 per cent. The slower the fiber the longer is this initiation time; it is presumed, though, that the percentage error due to it will be approximately the same for all fibers.

Not infrequently, in order to get a complete series, it is necessary to include the conduction rates of fibers whose potentials overlap somewhat at the lead. In such cases the beginning of the spike cannot be located with the customary accuracy. The error from this source cannot be over half the time to maximum of the spike, or, in the case of the fastest fibers, 0.2σ, about 3 per cent of the total conduction time. The extreme error in conduction time determinations in long nerves probably is never higher than 5 per cent; and determinations usually can be duplicated within 2 per cent. Conduction rates determined from two loci of stimulation not widely separated agree within 4 per cent. With wide separation between loci of stimulation the agreement is not quite so good and often wide discrepancies are seen. These are always in the direction of slower conduction in the more peripheral parts of the axon and may be referable to branching.

Conduction distances less than 20 mm. have not been used in determining conduction rates. In nerves as short as this electrode width and the difficulty in estimating the tension required to bring the nerve to its normal length introduce additional errors. The error for 20 mm. of conduction is probably not over 10 per cent, but relative conduction rates in such preparations probably are valid to 3 per cent.

Irritability by induction shocks. For stimulation with induced currents, break shocks from a Porter induction coil have been used (fig. 1). Under the conditions of the experiments, with the core in, the shock has a measurable duration of 0.06σ; with the core out 0.03σ. Except when otherwise stated shocks from the cored coil have been employed. Shocks resulting from interruption of the primary circuit in the manner described above, are sufficiently reproducible to allow an accuracy of 0.25 per cent in the expression of threshold induced voltages.

As a routine the secondary coil has been home and the induced voltage varied by a precision resistance, IR, in series with a five volt Edison primary battery. With a given primary potential the induced voltage varies practically directly as the primary current or inversely as the primary resistance. From readings of the precision resistance, relative thresholds may be expressed as calculated primary current, or the resistance readings may be taken directly as an expression of irritability. Under routine conditions, with the core in and secondary home, the most irritable fiber is stimulated by a primary current of about 0.5 ma. The make and break shocks are widely spaced temporally, and as the make shock is usually below the threshold of the fiber under observation its effect may be disregarded.

When necessary to balance out the shock artifact a 1000ω "noninductive" resistance is placed in series with the electrode proximal to the lead, and a 20,000ω "noninductive" potential divider connected across the coil terminals in parallel with the circuit formed by calomel cells, nerve and the 1000ω resistance. By grounding and adjusting the slider of the potential divider (see fig. 1) a partial balance in the circuit, *B2*, may be established according to the principles outlined by Bishop (1927), and the shock escape reduced to a negligible value. This modification of Bishop's balancing circuit has the advantage that changes in the balance produce only slight variations in strength of stimulation and in the physical constants of the circuit.

Owing to the spontaneous variability of fibers described above, thresholds cannot be measured precisely, and must be defined as the strength of shock that stimulates as often as it fails to stimulate. In the case of the most irritable fibers this point can be found with an accuracy of about 0.5 per cent; for the least irritable fibers the limit of accuracy is of the order of 10 per cent. Under certain circumstances an unusually marked treppe may reduce still further the accuracy of the readings by this criterion. In addition to this difficulty it is found that the relative irritabilities of axons may vary widely along the length of the nerve. To cite an extreme case, at one point on a fresh nerve the irritabilities of two fibers were to each other as 1:3, at another as 1:9. Such great differences probably are not entirely attributable to differences in the accessibility of the fibers to the applied current.

As a matter of routine determinations were made of the irritability *1*, of the most irritable fiber extending, in the plantaris preparation, from the stimulated point to about the point (the level of the knee) from which leads usually are taken in sciatic preparations, and *2*, of the most irritable fiber extending to the plantar nerve. In one preparation the most irritable fiber in the peroneal reached the plantar nerve. In other preparations the most irritable plantar fiber was the less irritable by from 2 to 62 per cent. Judged, therefore, by the irritability criterion and by conduction rate, the axons of plantaris are representative of the entire range found in the peroneal nerve.

If the axon threshold expressed as primary voltage be plotted against conduction rate the points derived from a nerve determine a curve of the hyperbolic type, concave upwards (fig. 5). In most cases the points fall quite regularly about the indicated curves, in others (curve D) rather irregularly. The scattering of the points is particularly noticeable with fibers conducting more slowly than 2 m.p.s. In part this probably is an expression of the damage done by the very high voltages required to bring the short shocks up to the threshold of these low irritability fibers, and, in part, possibly of greater frequency of branching. When composite

curves are made of the data derived from many different preparations these individual variations are seen to be random. Conduction rate plotted against threshold voltage, therefore, gives a curve without demonstrable discontinuities in any part of the range.

Irritability measurements by constant currents. Chronaxie. For the determination of the relation of current duration to strength and of chronaxie in axons a circuit (fig. 1) was devised by which constant currents of any desired duration, or condenser charges of any desired duration, and of known voltage could be applied to the nerve. The potential for this cir-

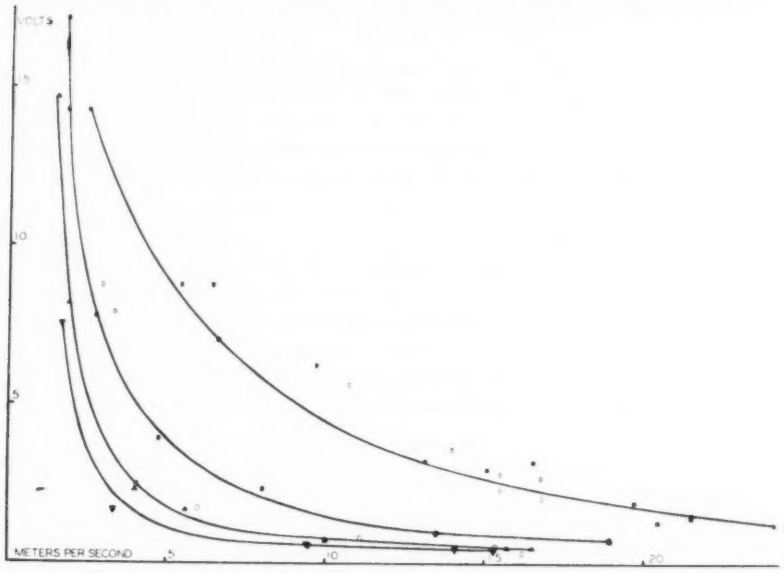


Fig. 5. The relation of the thresholds of axons to their conduction rates. Induction shocks. Data from six preparations. The D curve has not been drawn. Ordinates, relative volts.

cuit is supplied by heavy duty radio batteries, *CP*, through an 8 point switch with 4.5 volt steps. Intermediate ranges are obtained through a low resistance potential divider with a 5 volt range which may be read accurately to 0.2 per cent. For rapid determinations of chronaxie the voltage is doubled (switch *CD*) by leading from a similar point switch in gang, with 9 volt steps and by imposing 10 volts across the potential divider. When the switch, *CS*, is in the proper position a constant current is initiated by the closure of one key, C_1, and broken by the opening of a second series key, C_2. These keys may be moved independently and their temporal separation determined by direct observation of their electrical

artifacts on the face of the Braun tube. A selector switch, CS, removes the second series key, C_2, from the circuit and the same make key, C_1, initiates the charging of a calibrated variable condenser through the same resistances and to the same potential as with the constant current. A third key, C_3, serves to discharge the condenser, C, a half second later. The circuit is so designed that only 18 per cent of the total potential is required for stimulation of the nerve and the resistances are, therefore, of such an order that the physical constants of the system are almost wholly independent of the nerve circuit. From the capacity of the condenser and the resistance in the charging circuit, the RC product may be calculated. According to Lapicque (1926) and Monnier (1928), the RC product multiplied by the factor 0.37 gives the equivalent duration of a constant current of the same voltage. With the system employed the calculated values obtained by the condenser method approximated the observed constant current durations. As the capacity of the variable condenser may be more quickly read than the duration of the constant current subsequent determinations of chronaxies and of voltage-duration relations were determined by the former method.

Sakamoto (1933) by using a capillary pore electrode has succeeded in obtaining from the frog's sciatic the data needed for plotting eight smooth strength-duration curves of individual motor fibers, each one, presumably, from a different preparation. Other characteristics of the fibers were unknown. In a number of plantaris preparations, we have obtained the same information in each case from a number of fibers of known and widely differing conduction rates (Blair and Erlanger, 1933a).

In the first experiments chronaxie alone was determined, using essentially the method of Lapicque (1926) except for the use of calomel half-cells instead of Ag-AgCl stimulating electrodes and of the charge of a condenser instead of the discharge. It became necessary, owing to the inadequacy of the condenser method, particularly in the case of fibers of low irritability, to determine rheobase with the constant current. Typically (fig. 6) the fastest fibers (conducting at 25 m.p.s. at 25°C.) have an average chronaxie of 0.3σ. As conduction rate diminishes chronaxie diminishes regularly to reach about 0.2σ at about 10 m.p.s., and returns to its original value when the conduction rate has fallen to about 5 m.p.s.; it then lengthens very rapidly as conduction rate continues to fall, reaching a maximum of about 7σ in the case of the slowest fibers. The validity of these direct determinations of chronaxie was controlled by chronaxies read from complete strength-duration curves of axons (see below and figs. 7 and 8). The relation between conduction rate and chronaxie was the same by both methods.

These observations, therefore, fail to support the contention of the Lapicque school (Monnier and Dubuisson, 1931) that there is an inverse

relationship between the conduction rates and the chronaxies of nerve fibers. Indeed, the peculiarity of the relationship to conduction, differing as it does so radically from the relationship between conduction rate and

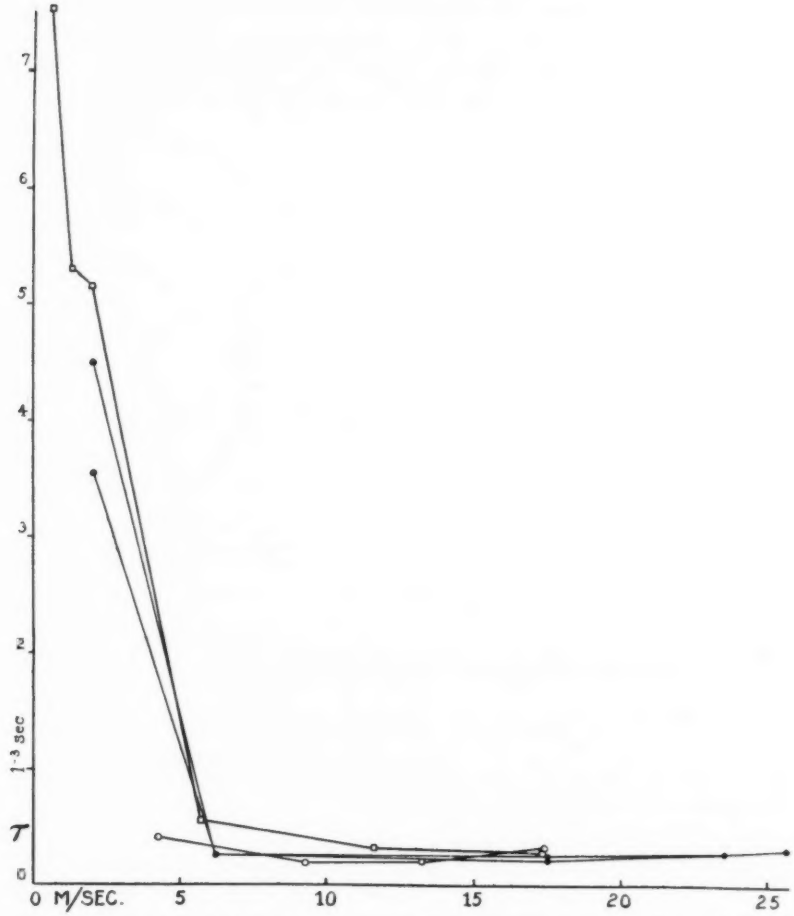


Fig. 6. Relation of chronaxie of axons to their conduction rates. Determinations from the same nerve connected by lines. The fibers conducting at about 10 m.p.s. have the shortest chronaxies. Ordinates, sigmas.

every other index to nerve activity we have investigated, casts serious doubt on the value of chronaxie as a measure of irritability.

Three factors, among others, have been shown to qualify chronaxie deter-

minations: the electrode (Davis, 1923), the external resistance (Eichler, 1931), and the length of the interelectrode span (Lapicque, 1926). Although in the present studies the physical electrodes are identical for all of the fibers of a nerve the effective physiological circuits (to and through the individual fibers) are not; each fiber has its own electrode, resistance and relative interelectrode distance. That these may be among the factors modifying chronaxie values at a point is indicated by the fact that determinations on the same fiber tend to be slightly longer with a decrease

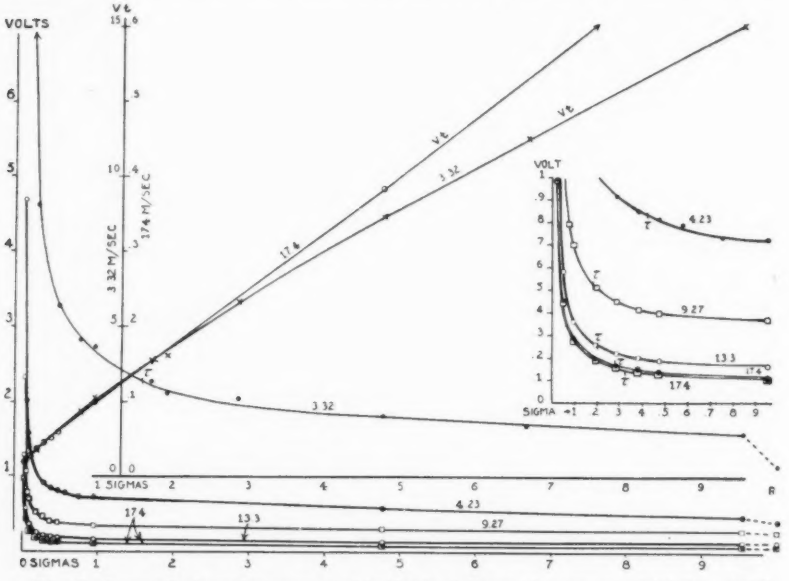


Fig. 7. Strength-duration curves of individual fibers of a nerve. 2/16/33. 22°C. Conduction rates indicated by numbers on the curves. The inset is the first unit of the coördinate system plotted on a larger scale to show the positions of the chronaxies (τ). The lines sloping toward zero in the added coördinate system are the Weiss law curves for the fastest and for the slowest of the fibers.

in the cross section of the shunting tissue. Furthermore, Grundfest (1932) shows that the chronaxie of a single fiber varies along its length. In how far these variable conditions, inherent in nerve structure, are responsible for the behavior of the chronaxie values in the present experiments, it is impossible to say.

Strength-duration curves of axons. Strength-duration curves derived from different tissues approximate in general the form of rectangular hyperbolas. On the basis of this resemblance, various investigators, notably Weiss (1901) and Lapicque (1926), have endeavored to derive

formulae expressing the relation of strength to duration which will be applicable in all cases. It has been difficult to compare strength-duration curves of different origins with each other and with calculated values because of the inability to obtain the necessary data under comparable conditions. For instance, data cannot be secured from motor nerve and from

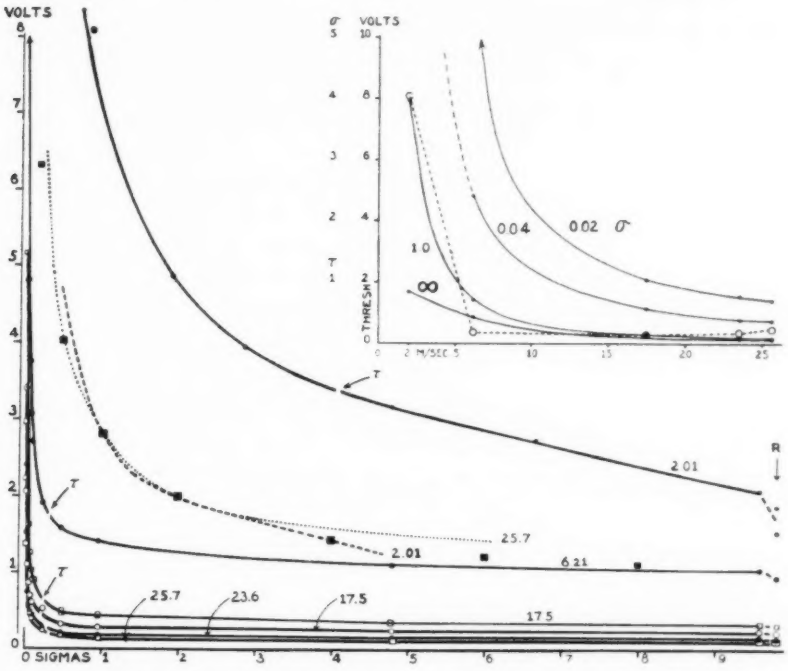


Fig. 8. Strength-duration curves of individual fibers of a nerve. 2/14/33. 22.8°C. Numerals indicate the conduction rates. Chronaxies (τ) marked by breaks in the curves. The squares are selected points from the canonical curve of Lapicque. The dotted and broken lines are the strength-duration curves of the fastest and of the slowest fibers of this nerve plotted on the same system of coördinates as the canon. Inset—A family of curves derived from the strength-duration curves showing the relation of conduction rates to threshold voltages with currents of different durations as indicated by the numerals on the curves. The circles are the chronaxies plotted with the same abscissae.

smooth muscle without modification of both the physical electrodes and the characteristics of the stimulating circuit. Neither can one be certain when dealing with different structures that they have been treated alike in other respects. But fibers in one and the same nerve have a reactivity range, as indicated, for example, by their conduction rate range, of

1 to 100; they are observed under identical experimental conditions involving no change of stimulating circuit, physical electrodes or question as to relative rheobasic values. The data thus secured are accurate within 2 per cent and, since nerves do not fatigue at the rate of stimulation used, remain constant for hours.

The strength-duration curves derived from the various fibers (figs. 7 and 8) are similar in configuration, but not identical. The dissimilarity is indicated by the fact that while rheobasic voltage has an inverse relation-

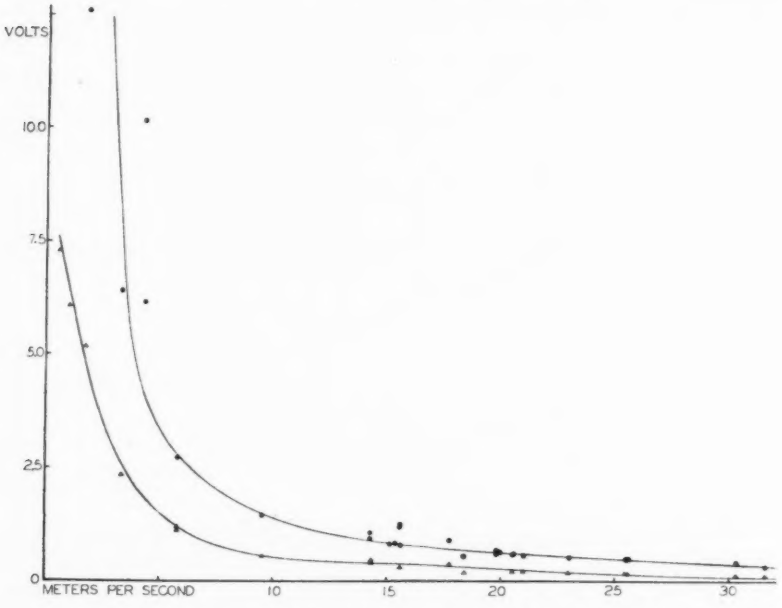


Fig. 9. Relation of thresholds to conduction rates of axons in a relatively large nerve. Stimuli, condenser charges. For the curve marked by triangles the condenser capacity was ten times that for the curves indicated by dots.

ship to conduction rate, chronaxie initially varies in the same direction as the rate (down to 10 m.p.s.) and then oppositely. Since the configurations of the curves are not identical, no formula based on the value of a single point (chronaxie) can express the relation of strength to duration.

Additional curves expressing the relation between conduction rate and threshold voltage can be derived by plotting against the conduction rate of the fibers whose strength-duration curves have been drawn the voltages of the constant currents of any given duration required to stimulate. In the insert, figure 8, are the curves drawn on the basis of constant current

durations of 0.02, 0.04, and 1.0σ and "infinite." The result is a family of smooth curves concave upwards and of the hyperbolic type. Naturally, the range of threshold voltage is narrowest with currents of "infinite" duration and increases progressively with decrease in the duration of the stimulating current. The chronaxies of the same fibers, derived also from the strength-duration curves, are plotted on the same graph in such a way as to bring out clearly the differences between the results of these two general methods of measuring irritability.

Two curves illustrating effectively the same result as the one depicted in figure 8 (inset), but from a nerve assuredly undamaged, and supplying

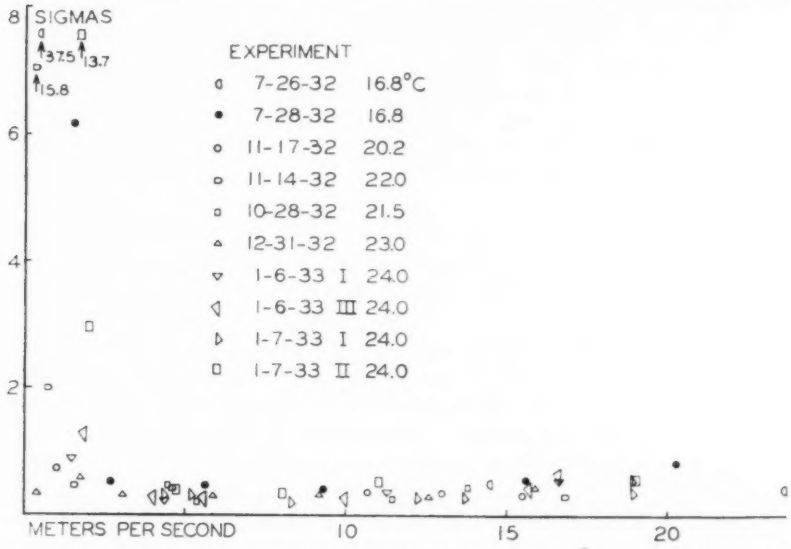


Fig. 10. Relation of summation intervals to conduction rates. Data from eight different nerves, in two of the preparations from two loci.

a sufficiently large number of fibers to control random variations, are shown in figure 9. They are based on determinations of the conduction rates and threshold voltages in a considerable number of the axons of a relatively large nerve with shocks derived in one case (dots) from a short condenser charge, in the other (triangles) from one ten times as long. All the curves expressing the relation between threshold and conduction rate of axons are free of discontinuities.

Summation intervals. The summation intervals of axons have been determined in a number of preparations by essentially the method previously described (Erlanger and Blair, 1931a). The sensitizing shock, 80 per cent of threshold, was from an air-cored coil excepting where, as occasionally

happened, it became necessary to insert the iron core in order to obtain shocks sufficiently strong to stimulate fibers of very low irritability. The results from ten of these experiments done, for the most part, at room temperature, are shown in figure 10. It is seen that as the conduction rate diminishes the interval shortens from about 0.4 to 0.5σ at 20 m.p.s. to about 0.3σ somewhere between 2 and 3 m.p.s. In all excepting one case it then lengthens, rapidly attaining durations as long, sometimes, as 70σ, in fibers conducting at rates slower than 1 m.p.s. Determination of the longer summation intervals is markedly interfered with by treppe but the results of this can be controlled. However, there are frequent fluctuations in the values for a fiber, far too wide to be assigned to experimental error. Since the chart includes all of the data of the experiments selected this fluctuation appears there. The number of determinations included is amply sufficient to care for this difficulty and to indicate the general trend of the mean. The duration of the summation interval at no time is less than five times that of the sensitizing shock; in the case of the fastest fibers it usually is about twelve times as long and of the slowest fibers hundreds of times.

There is no apparent simple relation between the summation interval and other manifestations of fiber activity. This result is of interest in view of the belief (Keith Lucas, 1910) "that the summation interval may be regarded as a measure of the rate at which the excitatory disturbance subsides." This view is based upon the observation, among others, that in the case of different tissues such, for example, as motor nerve, voluntary muscle and heart muscle, the summation interval increases in the order given which is also the order of the duration of their responses. In a comparison of different tissues, however, one has to consider not only speed of response and voltage threshold, but also the influence of both gross tissue differences and contact differences on polarizability. In the case of nerve one deals with fibers ranging in speed of response as widely in many respects as do nerve and muscle, but all exposed to the same conditions, and yet, excluding now fibers conducting under 2 m.p.s., summation intervals actually shorten as the reactivity of the fibers diminishes. The summation interval, therefore, does not vary with speed of response per se and in the case of axons, at least, there is no simple relation between them.

One might at first be inclined to account for the configuration of the summation interval curve on the basis of the presence in nerve of two types of fibers. There is not the slightest evidence of more than two. One type would include fibers conducting at rates over 2 m.p.s. and would be characterized by summation intervals that shorten slightly as conduction rate decreases. The slower fibers would make up the other type and would be characterized by summation intervals that increase, and very rapidly, in duration as conduction rate slows. This interpretation is

rendered improbable by the position of the discontinuity. According to Bishop and Heinbecker the most sudden alteration in the continuity of fiber properties lies between their B_1 and B_2 types. On that basis the break should, therefore, occur at about 4 m.p.s. Though evidence is lacking, we are much more inclined to attribute the result to some secondary action of the current on the irritable mechanism of the fibers; it must be this, or else the recovery speed of the excitable mechanism has no relation to its speed of action. It might be supposed, in explanation of our result, that just as constant currents, when increased in strength, at first enhance irritability and then depress it, so these subthreshold shocks, as their strength is increased, might very well at first facilitate a readjustment of some secondary polarization which they produce and later retard it, with the result that the nerve recovers its normal irritability more and more quickly at first and then, with still greater strength of shock, more and more slowly. Is it not possible that if as strong a shock could be applied to a fiber of high irritability without causing it to fire off, it, too, would exhibit a long summation interval? But irrespective of what might be its cause, it is the long summation interval of the fibers of low irritability that puts them in the class of so-called iterative nerves.

Be this as it may, the behavior of the summation interval does not necessarily signify that nerve is made up of two types of fibers responding characteristically to subthreshold shocks.

Refractory phase. The absolutely refractory periods of the fibers contributing to the α, β and γ waves were first measured in our laboratories at a time when the cathode ray oscillograph technique involved stimulation at the rate of 10 to 20 times per second. The values found in the bullfrog's sciatic were 1.42, 2.06 and 4.46σ, respectively (Erlanger, Gasser and Bishop, 1924). Later is was found (Erlanger, Gasser and Bishop, 1927) at times, especially with slower stimulation rates, that the differences in the refractory periods were so slight as almost to put them within the error of the method. When fibers of slower conduction were investigated each of the types recognized by Bishop and Heinbecker (1930) were found by them to have characteristic refractory periods, namely, A, 0.8–0.9 (in the bullfrog) and B_1, 0.9–1.1, B_2, 3.5–7.0, and C, 4.5–10.0σ (in the turtle), the higher values in each case being attributed by them to deterioration of the nerve. An analysis of the recovery process in the individual *fibers* of a nerve now shows that the rate of recovery at any stage bears an inverse relation to their normal conduction rates. In other words, the slower the fiber, the longer are its absolutely and relatively refractory periods.

The difficulties connected with the determination of relative recovery rates are manifold. A shock imposed on a resting nerve so modifies it as to cause a response. In addition, previous studies (Erlanger and Blair, 1931a) have shown, a shock affects active nerve by changing the curve of

returning irritability. A strong, though at the time, subthreshold, shock delivered during the refractory phase, it was shown, hastens the return to normal at its anode. And in the course of the present investigation it has been found that such a shock produces the reverse effect at its cathode, prolonging the recovery phase. The significance of this effect is demonstrated in figure 11.

Relatively refractory periods are usually determined by producing a response to an initial short shock, adjusting a second similar shock so as to be just threshold for the response, and then approximating the two temporally until the second response fails. This temporal separation of the

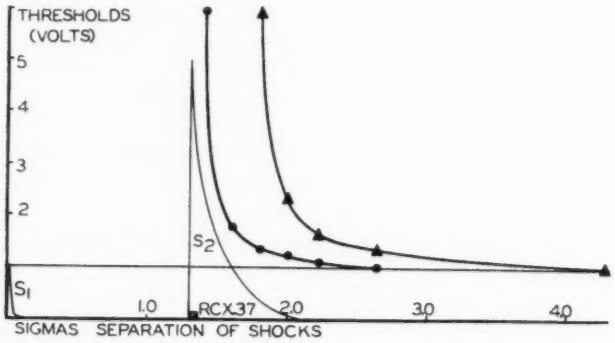


Fig. 11. Effect of a cathodal shock, delivered during the absolutely refractory phase, on the recovery curve of a single fiber (conduction rate 11.5 m.p.s.). 3/21/33. 23.7°C. Ordinates, threshold voltage in terms of normal fiber threshold; abscissae, separation of initial, S_1, and testing shocks. Dots, normal recovery curve following response to S_1. A short cathodal condenser charge, S_2, (stimulating equivalent, 0.06 σ constant current) adjusted to five times the normal threshold, indicated by the horizontal line (or fifteen times threshold of the most irritable fiber of the nerve), is imposed during the absolutely refractory phase and the recovery curve redetermined (triangles). Short, strong cathodal shocks prolong the recovery curve in all its phases.

two shocks has been taken to represent the end of the relatively refractory phase, and the curve of recovery has been determined by plotting the threshold voltage of the testing shock against the separation of the two shocks. The absolutely refractory period has been determined by increasing the testing shock to five times threshold and approximating the two until the second response fails. When, as has been the usual procedure in this laboratory, the two shocks are delivered through the same pair of electrodes, the effect of the initial shock may persist after the first response, and modify recovery. That this happens with very strong initial shocks is demonstrated by a comparison of the recovery curves of single fibers

based first on the results obtained with the two shocks delivered through the same pair of electrodes, and then through different electrodes with the testing shock proximal to the lead. In all cases, the recovery time determined by the first method is the longer. With relatively low voltages no such effect can be demonstrated, even when the shocks are sufficiently long to stimulate fibers of low irritability.

Similarly the effect of the testing shock acting through the utilization and latent periods may modify recovery time. By analogy with simple responses, latency should be expected to increase with an increase in threshold during the relatively refractory period, thereby giving a longer time for the testing shock to produce an effect on the recovery before the

TABLE 1

Comparison of results obtained in refractory period determinations

Plantaris, 24-hour preparation, 24°C. Sensitizing coil air-cored, testing coil iron-cored.

CONDUCTION RATES	THRESHOLDS (RELATIVE)	SEPARATION OF THE TWO SHOCKS, IN SIGMAS			
		Point I, 104 mm. conducting distance		Point II, 85 mm. conducting distance	
		Testing shock		Testing shock	
		5 × Threshold	Threshold	5 × Threshold	Threshold
m.p.s.					
18.9	100	0.89	1.90	0.92	2.24
18.9—	104		1.90		
18.9—	104		1.78		
12.2	116	1.08	2.01		
13.7	120		2.49	1.04	2.43
8.32	305	1.45	2.76		2.76
5.20	513	4.39	3.46	7.00	6.70
2.01	1940	9.05	6.83	7.87	6.15
1.83	2410	8.55	8.00	7.87	12.00

second response develops. That this happens is shown by the disappearance of a consistent threshold response of a low irritability fiber stimulated during the relatively refractory period by a strong, short testing shock, when this shock is still further increased in strength. Indeed, when the strength of a testing shock from a coreless Porter inductorium is increased to five times threshold for the purpose of determining the absolutely refractory period of a fiber of low irritability the recovery time usually increases so that the second response disappears at a shock separation that is as great as, at times even greater than, the determined relatively refractory period. The durations, therefore, of the absolutely and relatively refractory periods of a fiber, determined by such a method, may be alike (see table 1). In view of this fact, and of the irreversible damage which very

strong, short shocks produce in all fibers, such shocks are impractical for the investigation of refractoriness. With longer shocks, though, while the longer utilization and latent periods provide a longer period for effective polarization, the relatively low voltage required reduces the necessary cur-

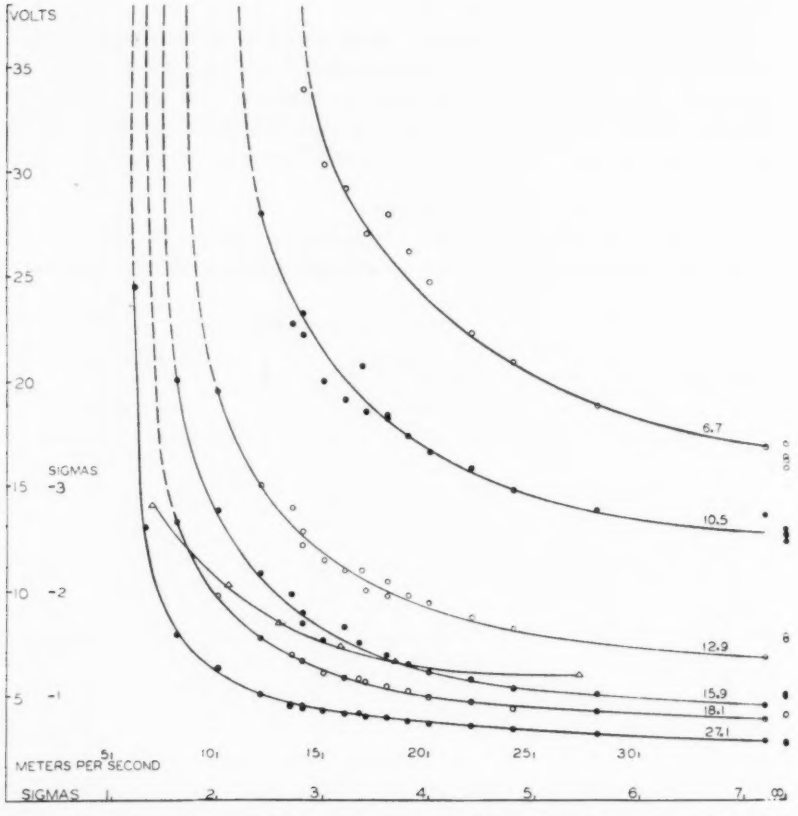


Fig. 12. Recovery curves of 6 axons of a nerve with conduction rates given by the numerals on the curves. Ordinates, thresholds in relative volts; abscissae, σ.

For the curve marked by triangles the ordinates are the absolutely refractory phases of the fibers determined by the extrapolations, the abscissae, the conduction rates of the fibers.

rent to such an extent that there is no demonstrable effect on the recovery curves even when the strength is increased some fifteen times the threshold of the most irritable fiber. It must be pointed out, however, that in the case of the slower shocks the relatively longer utilization and

latent periods associated with the initial and testing shocks probably are not exactly the same, and, therefore, the separation of the two shocks is not an entirely valid index to the duration of refractoriness.

Figure 12 presents the recovery curves of single fibers in a typical experiment at 21.3°C. Threshold voltages are plotted against separation of the two shocks. Stimulation was by a pair of condenser charges, each adjusted to have a stimulating value equivalent to a constant current of 0.25σ. The rate of stimulation chosen, once in four seconds, was such that the threshold of the least irritable fiber, as shown by the response to the initial shock, remained constant. The nonpolarizable stimulating electrodes were placed on the unbranched portion of the sciatic, and the axon spikes recorded from the plantar nerve. The voltage of one charge was set just above the threshold of the least irritable fiber under observation, and the key arranged to close coincidentally with the initiation of the spread of the time line. The key controlling the second charge was moved to give different separations of the two shocks and their temporal separation determined (see determinations of conduction times). With each separation the threshold voltages were determined for the six individual fibers. Readings were scattered both temporally and as to order of determination. At frequent intervals the thresholds with infinite separation of the shocks were determined.

It is seen *1*, that as the conduction rate (indicated by the numerals above the curves) decreases, the thresholds ("infinite" separation) increase as previously described; and *2*, that the recovery curves, as indicated by the voltage thresholds of the different fibers, are of a similar hyperbolic type, the recovery time increasing in continuous series with increasing threshold and decreasing conduction rate. To avoid complications due to polarization the maximum voltage was not carried beyond 13 times the threshold of the most irritable fiber or hardly twice that of the least irritable one. As previous work has indicated a supernormal phase may or may not be present. Of the six fibers, those conducting at the rates of 18.1, 15.9 and 12.9 m.p.s., show supernormal phases, indicated by a lower threshold with 7σ separation of stimuli than with "infinite" separation. As with the strength-duration curves, single points have little meaning here; the end of the recovery phase of fibers having no supernormal phase cannot be determined with any accuracy because of the asymptotic character of the curves; and the values thus found can be in no way comparable with the readings secured by employing the sharp beginning of the supernormal phase, when it is present, as the sign of the end of the recovery phase. Determination of absolutely refractory phases by increasing the shock to 3 or 5 times threshold and decreasing the separation of shocks until no response is obtained leads to error because of the effect of polarization by the testing shock on recovery.

From the curves, absolutely refractory periods were approximated by extrapolation (broken lines) and the values thus obtained (triangles), plotted against conduction rate on the inside coördinate system of figure 12 (small print). This curve, as well as those from other experiments, is without discontinuities.

Experiments have not been performed specifically for the purpose of determining the relation of the absolutely refractory periods to the temporal configurations of the axon spikes (see below). Comparison of the two values as obtained in different preparations under conditions not always exactly comparable, shows that the refractory periods are at least 2 to 3 times as long as the crest times of the spikes throughout the entire range.

Amplitude and temporal configuration of axon spikes. From favorable preparations of plantaris it rarely is possible to obtain more than four to six spikes separated cleanly and, therefore, suitable for measurement of height and configuration. This number is insufficient, particularly in view of the many exceptions to the rule, to define a curve.

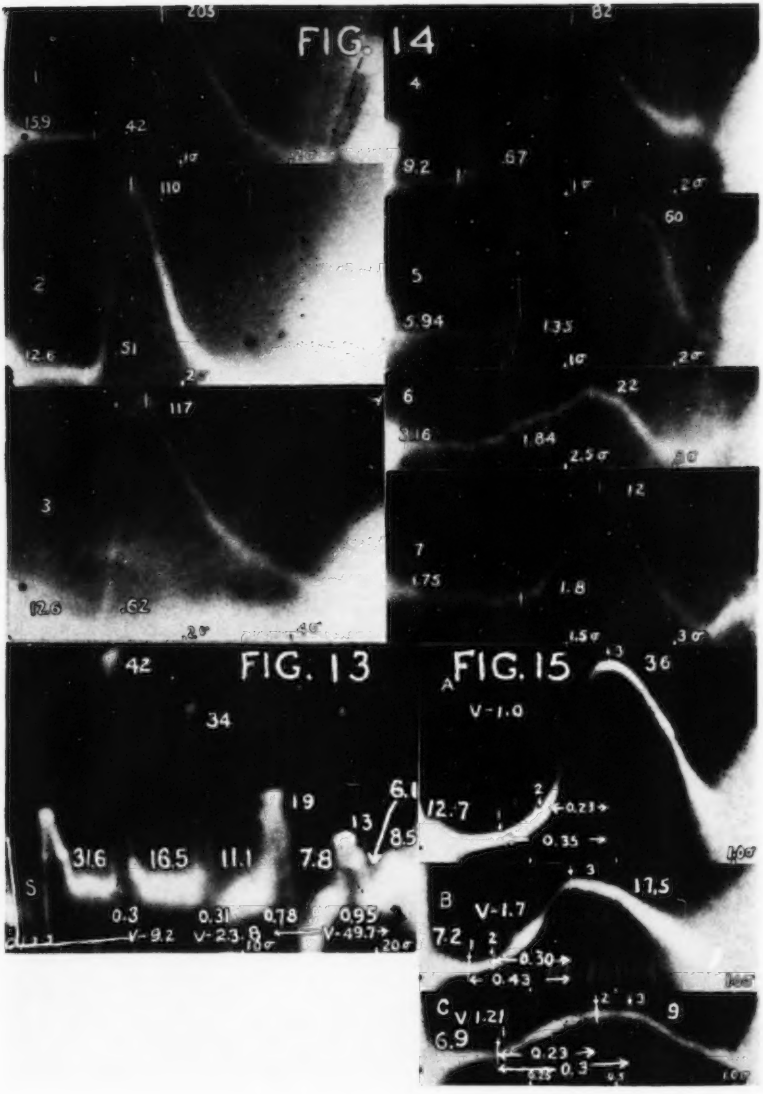
Relation of amplitude to conduction rate. In general one finds for each nerve that the slower the rate of conduction the lower is the spike in terms of microvolts. Illustrations of this relationship in individual nerves are seen in figures 3, 13, 14 and 16. The maximum recorded amplitude from fibers in small nerves has been of the order of 400 μv. In order to obtain more information regarding this relationship, for each of a number of nerves with differing absolute values an average amplitude for fibers of the same conduction rate was assigned a single representative axon spike and in each case this value (circles in fig. 17) has been used as a base for the expression of the relative amplitudes of the other spikes of that nerve. Justification for this method of treatment is derived from the assumption that axons conducting at a given rate under the same conditions develop the same potential. When the data from the different experiments are thus correlated and plotted on the same system of coördinates, the points fall, though rather widely, to be sure, about a straight line. There is no

Fig. 13. Contact print showing 5 cleanly separated axon spikes. 4/17/33. Conduction distance 129 mm. 24.3°C. S is the shock artifact. V in this and in subsequent records is the threshold. The coördinate system, shown by the lines ruled in the lower left hand corner, is not squared with the page.

Fig. 14. Series of axon spikes from a nerve refrigerated for 24 hours. 12/31/33. 23°C. Number 7 is probably cut short by the diphasic artifact. A comparison of *3* and *5* demonstrates the difficulty of obtaining comparable measurements of crest time from spikes of different configurations.

The conduction rates and basic irritabilities of the fibers producing spikes *2* and *3* were identical. It was possible to obtain separate pictures because sometimes one spike, sometimes the other, appeared alone at the lead.

Fig. 15. Axon spikes of three fibers in a skin nerve. Amplification and speed same for all. 1/25/33. 24.7°C.



Figs. 13–15

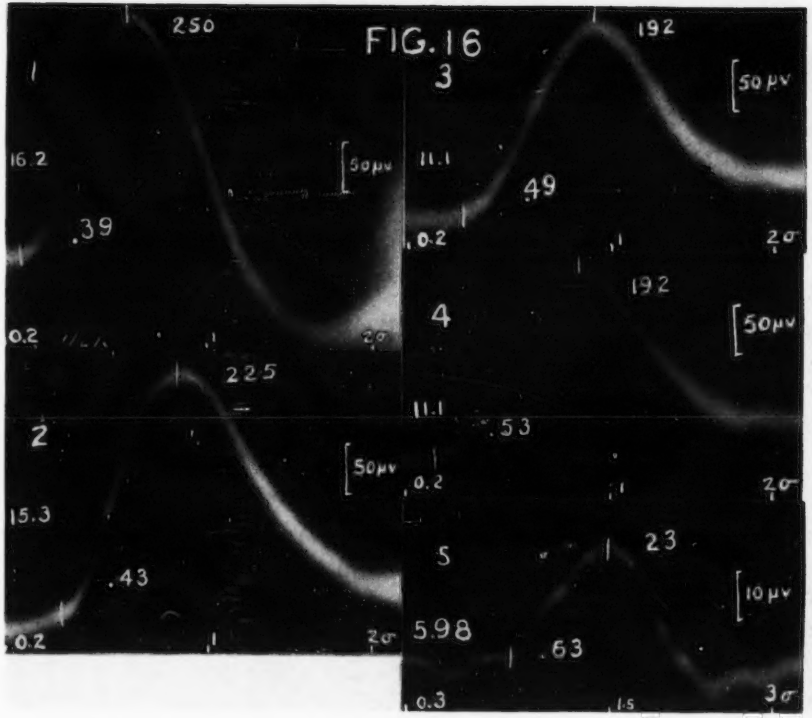


Fig. 16

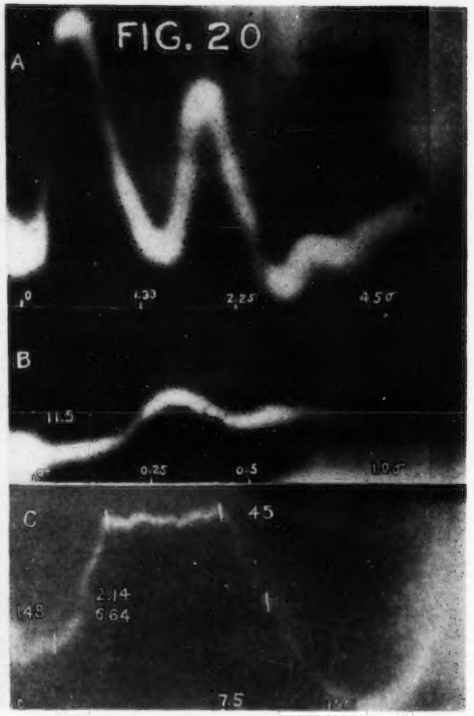


Fig. 20

Fig. 16. Series of axon spike records from a fresh nerve. 1/16/33. 24°C. *1* and *5*, diphasic, the latter distorted by noise. *2*, *3* and *4*, typical spikes.

Fig. 20. Atypical spike records. *A*. Spikes from three unlike daughter axons resulting from stimulation of the single high irritability parent fiber. The third spike lies in the diphasic artifact of the second. *B*. Similar record, but with lower irritability parent axon. There seem to be three daughter branches. *C*. High amplitude (45μv) spike of slow conduction. Possibly the result of multiple branching near the lead. First figure under the wave, end of rising phase; second, beginning of falling phase. A type encountered not infrequently.

evidence of any discontinuity in their arrangement. The meaning of this result in relation to fiber diameter and conduction rate is considered in the discussion.

Relation of time to maximum to conduction rate. In individual nerves crest time in general increases as conduction rate slows, though, as in the case of amplitude, there are many exceptions to the rule. We are confronted here, however, with the difficulty that there is no perfectly valid way of correlating the data from different nerves. Our procedure, there-

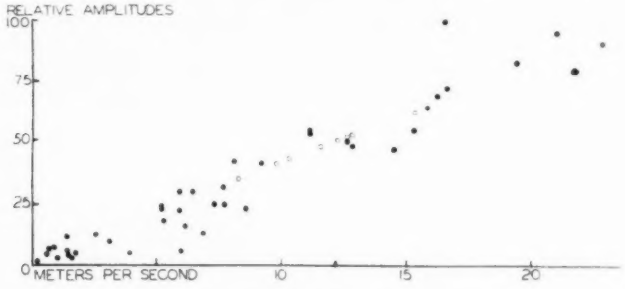


Fig. 17. Relation of the relative amplitudes of axon spikes to conduction rates. For method of construction see text. The points appear to fall about a straight line (see fig. 21).

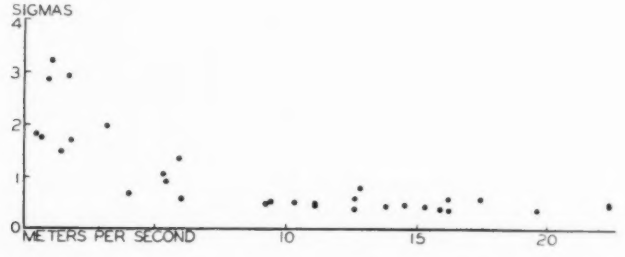


Fig. 18. Relation of time to maximum of axon spikes to conduction rate. Room temperature. The points indicate a curve of the hyperbolic type (see fig. 21).

fore, has been simply to plot on the same system of coördinates the data from a number of experiments performed under reasonably comparable conditions (20 to 25°C.). The curve indicated (fig. 18) is of the hyperbolic type; the slower the fiber the longer is its time to maximum. The time range in plantaris has been from about 0.3 up to about 4.0σ. The curve is without recognizable discontinuities.

Falling phase. The asymptotic approach to the base line of the falling phase of the spikes and the influence of diphasicity upon it stand in the

way of even reasonably accurate measurement of this part of the spike. The topic may be dismissed with the statement that the falling phase in general seems to increase as conduction rate slows.

Discussion of the difficulties in the way of comparison of configurations. *a.* Nerve diameter, as a factor affecting the recorded height of axon spikes, is a difficulty to which reference has already been made.

b. The thickness of the nerve also has a definite effect on the configuration of the axon spikes, as may be seen in figure 19, *A* and *B*. Nerve thickness may affect the picture in several ways: *1.* The axon spike from the larger nerve, *A*, has a more distinct foot; *2*, sometimes there is a distinct angle, as at the point 2, figure 15, *A*, where the foot joins the steeper rise; *3*, and where this angle is marked the foot may even be slightly convex upwards. The factors determining these three pictures are not at all

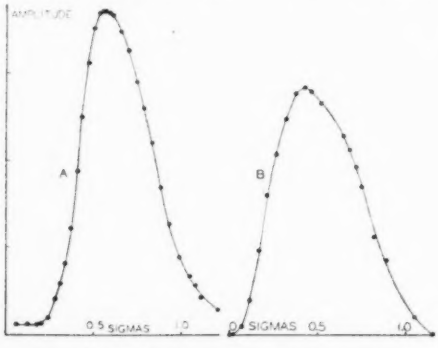


Fig. 19. Axon spikes redrawn in linear coördinate systems. *A*, is record A of figure 15 (from a relatively thick nerve). *B*, is record *1* of figure 14 (from a slender nerve). Amplitude is without significance.

clearly understood. With respect to *1*, it is possible that in thick nerve the foot, which presumably is due to spread of potential from the active region, is merely relatively higher with respect to the recorded spike potential than in thinner nerve and becomes prominent because the amplification is increased in order to bring the height of the axon potential record up to that obtained from small nerve. *2*. The angle that sometimes appears between the foot and the quicker rise possibly is to be attributed to some condition which in effect puts the lead off of the path of the active axon (Bishop and Gilson, 1929). This possibility is supported, and *3*, light shed on a possible explanation of the occasional convexity upwards of the foot, by the fact that in one experiment, by shunting plantaris with a large nerve, a normal axon potential was converted into one preceded by a positive wave. The axon potentials recorded by Matthews (1929) from the median peroneal nerve frequently exhibit such a preliminary positive wave.

The question that concerns us here is how to take the foot into consideration in measuring the records of axon potentials temporally. The total time to maximum of spikes measured from the beginning of foot to crest in large nerves where the foot is conspicuous is of about the same order of magnitude as the time to maximum in plantaris where the foot often is not obvious and cannot be distinguished from a more rapid rise. If the foot is due to spread of potential it should not be included in the time measurements and has not been where it has been possible to recognize it; but it is not possible to exclude it in the case of plantaris axon potentials. Even where the foot is fairly obvious (fig. 15, *A*) it is difficult to define exactly the time to maximum. If the end of the foot indicates the time of arrival of the active process at the lead the ordinate values of figure 18 are all too large.

Exceptional configurations. Exceptional configurations not infrequently account for unusual values for height and duration. Some of these unquestionably are to be accounted for by damage in preparation. In such cases further changes frequently develop in the shape of the spike, and the refractory phase at the lead is apt to be abnormally long as evidenced by occasional failure of the response to reach the lead. The data obtained from axons behaving in this way have been discarded.

But exceptional spike configurations are by no means all referable to damage, for atypical configurations are seen also in records from skin nerves. The pictures obtained from one and the same fiber in such a nerve fluctuate somewhat in height and in configuration, but scarcely more than can be accounted for by noise and variations in the lead. But the pictures yielded by different fibers of the same nerve are apt to have characteristically different configurations. Figure 15 shows contact prints of three different axon spikes from a skin nerve with the same leads, amplification and time axis. Spike *A* has a slowly rising initial phase, with possibly a faint indication of an angle, *2*, and a relatively sharp crest; *B* has a sustained falling phase showing an elevation; *C* has a sharp start, and an indefinite crest between *2* and *3*. These particular variations are not referable to differences in amplitude because they are quite as apparent when the amplification is adjusted so that the different spikes have the same amplitudes. Since the nerve is not damaged at the lead and is uniform in diameter, the differences must be referable to unlike active processes, to uneven sheaths, or to differences in the relation of the electrodes to the fibers. In the absence of evidence, our inclination is in the direction of assigning them rather to anatomical factors, such as sheath thickness and to the relation of the lead to nodes, than to inherent differences in the local process.

A much wider range of variation has been seen in plantaris preparations, possibly because they have been subjected to a more thorough scrutiny,

though, as has been stated, damage may be a factor here. A variation which has been observed on several occasions is illustrated by *3*, figure 14; the spike is too high and too long in relation to companion potentials. The maintained falling phase gives the impression that the fiber is damaged, but the amplitude is greater even than normal. In one experiment on a skin nerve, a group of similar fibers of intermediate irritability (about 10 in number) had spikes which were regularly double-crested, while all other spikes were normal in configuration.

Branched axons. Perfectly, or not quite perfectly, superimposed potentials from branches of a single parent axon may lead to error in the determination of spike configuration. When the superposition is imperfect, as in figure 20, *A* and *B*, the condition is easily identified. Multiple branching near the lead might produce much more complicated pictures; possibly this is the explanation of record *C*, an exemplar of a configuration seen not infrequently.

Fusion of similar spikes. Two fibers with identical irritabilities, conduction rates and spikes may fuse to form smooth curves of the usual configuration but with a higher voltage than that expected of a single response from a fiber of corresponding irritability.

Diphasic artifact. When the diphasic artifact is marked its effects upon the height and shape of the axon potential can be discounted, but not its unrecognizable effects. The limitation set by the amplifier input resistance makes it impossible in the case of small nerves to record brief potentials with the two phases cleanly separated even if there were available a sufficient length suitable for the purpose.

Demarcation potential. To reduce the diphasic artifact the nerve must be freshly killed proximal to the grid electrode, and injury modifies the amplitude of spikes recorded even as far away as 4 or 5 mm. It is quite possible that this uncontrolled influence varies not only from nerve to nerve, but differentially affects axon responses.

Amplifier noise. The relatively greater shunting in the case of slowly conducting fibers so reduces their spike amplitudes that the inherent noise of the amplifier produces significant distortion. In such cases the configurations can be ascertained only by a comparison of several records and then, often, only roughly.

Summary. The complications in the way of determining the amplitude and the time to maximum of the axon potential have been reviewed in part for the purpose of indicating that a certain amount of scattering of the points on the graphs correlating these with conduction rate is inevitable. In support of the plotted data it may be said that the experiments in which records were made and which serve as the basis for the data in the graphs form only a small fraction of the total performed. There are available over 200 additional experiments done with the other ends of this investigation in

view. In all the behavior of the axon potentials observed on the screen has been in complete agreement with the quantitative observations used in this section.

THEORETICAL CONSIDERATIONS. *Relation of fiber diameter to conduction rate.* If it be assumed that the intrinsic potential of action attains the same amplitude in all fibers then, if the whole nerve acts as a homogeneous shunting resistance, the measured potential, Y, would vary as the cross areas of the fibers, or as the squares of their diameters, D, (Gasser and Erlanger, 1927). But experiment indicates that the amplitude, Y, of the recorded axon potentials varies directly as the conduction rate, X. It, therefore, follows that the conduction rate varies as the diameter squared. Expressed with symbols:

$$Y = CD^2$$

and

$$Y = C'X$$

hence

$$D^2 = C''X$$

Gasser and Erlanger, however, came to the conclusion that "to a first approximation the velocity in the fiber is determined by the diameter." The basis for this conclusion was the reasonably close resemblance of theoretical reconstructions of the action potential waves to the actually recorded waves. The fibers they measured for this purpose extended down to 5μ in diameter and included only those contributing to the A wave. Later, when the potentials of fibers of slow conduction were found, Erlanger and Gasser (1930) extended the analysis to include all of the myelinated fibers. The latter reconstructions exhibited another elevation but it fell considerably earlier than the B elevation of the record from the same nerve, and it was, therefore, concluded that "the velocity of conduction in the small fibers is slower than the one calculated on a size basis from the velocity of the fastest fibers."

Recalculation of the data of the one experiment of which all of the necessary data are available (fig. 9 of Erlanger and Gasser, 1930), on the basis of proportionality of conduction rate to the cross area of the fibers (diameter squared), throws the B wave into just about the position it occupies in the record, but the reconstructed α, β and γ waves of A then fall somewhat too late. The deviation is not sufficient, in our opinion, to invalidate the applicability of the diameter squared rule to this portion of the action potential. But to decide a question fraught with such a large experimental error and based upon so many assumptions a much more careful consideration will be required than we have been able to give it thus far. It is

felt, however, that the evidence is not definitely opposed to the possibility that all of the fibers of the nerve conduct at rates which roughly are proportional to the squares of their diameters.

Relation of axon potential amplitude to fiber diameter. Through an argument similar to that employed above one derives the equation

$$\text{Amplitude of action potential} = CD^2$$

In other words, the amplitude of the action potential also is proportional to the diameter squared.

One might use the data on the time to maximum of axon potentials to work out still other interrelationships. This development, however, will be postponed until the opportunity comes to obtain records of the spike potentials on a linear time line, when it will become possible to obtain the slope of the ascending limb without having to replot the curves on Cartesian coördinates.

Discussion. Although there are many observations on record of responses of single axons, there is, so far as we know, only one other contribution to the general subject of this paper. Matthews (1929), employing his oscillograph, has determined the conduction rates and recorded the potentials of cutaneous and proprioceptive axons in the frog's sciatic. He found the two functionally different axons to conduct at rates around 12 and 16 m.p.s, respectively (ca 13°C.), thus confirming the conclusion reached by Erlanger (1927) that proprioceptive fibers are among the fastest of the sensory fibers. His crest times are longer than those reported here, probably due to inertia, but the relative times agree fairly well with the present data.

We have next to consider more fully the differences between our and Bishop and Heinbecker's points of view. Apparently they recognize histologically four general types of fibers, A, B_1, B_2 and C, (although they leave open the possibility of transitional varieties) with properties which are more or less characteristic, the less characteristic being conduction rate and irritability, the more characteristic duration of spike potential and especially the duration of the refractory period, the most abrupt change in properties occurring between the B_1 and B_2 groups where the differences in the case of some of the measures employed is "in the ratio of least 4 to 1." (Bishop and Heinbecker, 1930.)

We have not concerned ourselves with histology. However, the opinion seems to be growing among histologists that there is every gradation between heavily myelinated fibers and completely naked axis cylinders (Verzár, 1932). Our findings are in keeping with that opinion, in that by every measure we have applied we find that the properties of the axons vary in a perfectly continuous manner from one end of the range to the

other. The two apparent exceptions, namely, chronaxies and summation intervals, do not fit the scheme of Bishop and Heinbecker, for the sudden discontinuities that occur in the curves occupy different positions, neither of which lies quite in the B_1-B_2 gap of Bishop and Heinbecker.

Figure 21 visualizes our and what we take to be their points of view. The properties of fibers, as we have measured them, with conduction rate as the base, are represented as continuous curves (indicated by large type). In the same system are blocked out the comparable values for the A, B_1, B_2 and C types of fibers as given by Bishop and Heinbecker (small type). Their four blocks form extended points on, or not far from, our curves. The blocks furthest removed from their corresponding curves are those representing Bishop and Heinbecker's crest time values. The reason for this divergence will be considered in a subsequent paper.

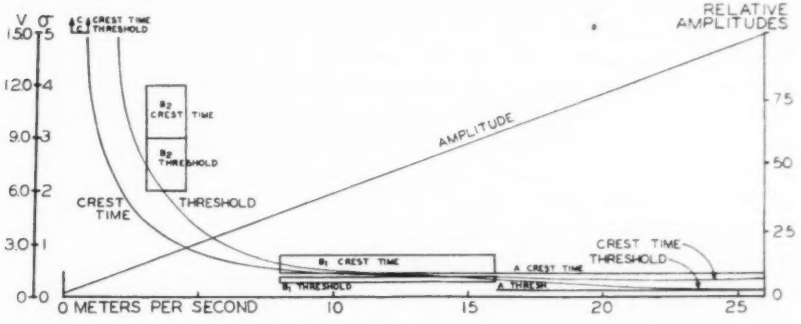


Fig. 21. Graph of the relation of threshold, amplitude and crest time to conduction rate (see text).

In their latest publication (1933)[3] Bishop and Heinbecker say that our differences are quantitative ones; that "if one reads 'groups' in place of 'types' . . . . these would be unimportant." With this we, indeed, agree if they consider their "axon types" as aggregations of fibers with similar properties from out of the general mass of fibers whose properties range continuously along smooth curves as described in this paper.

It may be of interest to direct attention to the bearing of the concept of a numerical grouping of fibers with a continuous range of properties on the development of certain phases of our knowledge regarding the constitution of nerves. The first observations with the cathode ray oscillograph, made when the effective amplification was 10 mm. per millivolt, disclosed three elevations in the continuous maximum conducted action potential of the frog's sciatic, very rarely a fourth (Erlanger, Gasser and Bishop.

[3] See also Blair and Erlanger, 1933. Proc. Soc. Exp. Biol. and Med., xxxi. 127.

1924). For the sake of convenience these were designated α, β, γ and δ. Later (Erlanger and Gasser, 1930), when amplification was increased so that 1 mv. deflected the spot 100 mm., two additional elevations, B and C, of the continuous potential were described, but then Erlanger and Gasser failed to find the δ wave. Even with the high amplification now available the present authors do not find in the frog's sciatic a discrete elevation corresponding with δ's position. Simultaneously Bishop and Heinbecker (1930) described in the action potentials of certain autonomic nerves the elevations designated by them, A, B_1, B_2 and C. Later (1931) they say that a potential with the properties "of the original delta wave" or B_1 "is invariably present in the sciatic of the frog," and that "for reasons not apparent" they "fail to support the position of Erlanger and Gasser in which they now deem it *expedient*[4] to disregard the delta wave. . . . ."

From the present standpoint it is of no importance whether or not the sciatic action potential presents a *wave* in the B_1 situation. The point we wish to make is that such *potential* as there may be in that position is produced by fibers all of whose properties, we believe, are known if their conduction rates, for example, are known. Erlanger and Gasser never regarded the region in the conducted potential of the frog's sciatic (or of any other large nerve) between A and B as free of potential, nor, as a matter of fact, the region between B and C (see their fig. 3, 1930); but they did not regard the A to B region as any more worthy of a special name than did Bishop and Heinbecker the region between B_2 and C.[5]

In our opinion the emphasis that has been placed on specific *elevations* or *waves* or *types* has had the effect of obscuring correlations which otherwise would have been obvious, and has given rise to certain unwarranted inferences both on our part and on the part of others.[6] When the statement has been made by workers in this field that a given elevation on a conducted action potential is made by fibers devoted to a given function (and to be specific, let us say to voluntary motion) it was never intended to mean that all of the motor potentials are confined to that elevation. That they are not confined to the α wave, but actually contribute to a very extensive stretch of the record, was clearly recognized, and proved by the analysis of the sizes of the fibers in a motor root that is free of sympathetic fibers, such as VIII in the frog (see fig. 8*a*, Gasser and Erlanger, 1927). The sizes of the fibers in motor VIII range from 20μ down to 2μ or even less, though there are very few so small. On the diameter squared basis the

[4] Italics ours.

[5] Those interested in following this matter further should compare the region between γ and B in figure *1d* of Erlanger and Gasser (1930), making due allowance for noise there, with the part of Heinbecker's figure 1 (1930) labelled B_1, making due allowance in this case for the diphasic artifact of the A wave.

[6] The writers assume entire responsibility for the opinions here expressed.

conduction rate range of these fibers would be from, say, 42 to about 1 m.p.s., on the basis of proportionality to diameter, 42 to 4.2 m.p.s. The fibers mediating other functions presumably range as widely in their conductivity. Concerning this, however, we know less, and want to direct attention to but one aspect of it, one with which we have had some experience, though still only in a qualitative way.

In sufficiently small skin nerves of the frog it is possible to determine the physiological properties of every one of the constituent axons. In any one such nerve the grouping of the axon potentials after conduction is perfectly constant but the pictures from the different nerves differ strikingly one from the other. The main point of interest at the moment, however, is the conduction rate of the fastest fiber. This, at comparable temperatures, has ranged all the way from 22 to 6 m.p.s. It seems justifiable to conclude, therefore, that the conduction rate range of the fibers of the skin sense that is conducted fastest is wider than from 22 to 6 m.p.s. In large skin nerves, it should be added, the variations exhibited by the small nerves are smoothed over and their action potentials, therefore, have a configuration that is more or less typical (Erlanger, 1927).

While there is every reason for believing that the conduction rates of fibers mediating a given function often are clustered in such a way as to make it possible to recognize their potentials by an elevation in the conducted action potential of a large mixed nerve, there nevertheless is evidence, such as that given by skin nerves, indicating that in different small nerves and presumably, therefore, in branches of a large nerve, the partition of the fibers may differ to such an extent from that obtaining in the parent nerve as to throw the elevations they produce into very different relative positions.

To cite an instance— when the sciatic nerve of the frog is stimulated the resulting action potential led from a connecting sensory root presents three elevations which have, and rightly, we believe, been regarded as the equivalents of the α, β and γ elevations in the sciatic (Erlanger, Bishop and Gasser, 1926). However, the specific configurations of the two sets of waves are very different (compare fig. 2, Erlanger, Bishop and Gasser, 1926 with fig. 2a, C and D, Erlanger, Gasser and Bishop, 1924). And when the conduction rates of the apparently corresponding waves are determined by measurements made in two ways, namely, from the stimulating electrodes on the peripheral end of the sciatic *1*, to a lead just peripheral to the posterior root ganglion, and *2*, to a lead on the central end of the root, the results obtained are not alike. At the time this subject was being studied no obvious explanation of these observations occurred to us. Now the differences can be attributed to a numerical grouping of the sensory fibers in the root that differs from that obtaining in the nerve.

The difficulties encountered in the search for the source of the fibers

making the B wave of the mammalian saphenous nerve can be accounted for in a similar manner. The B wave in the saphenous is an outstanding feature (see fig. 6, Erlanger and Gasser, 1930). It was natural to suppose, therefore, that it is formed by the potentials of sensory fibers. But we failed to find in mammalian sensory roots a "wave consistently occupying the position of B." Examination of the root records, however, (for example, fig. 12c, Erlanger and Gasser, 1930) discloses an abundance of "potential" between the A and the C waves. Furthermore, in records obtained by stimulating an appropriate root and leading peripherally from mixed nerve in the cat "nothing resembling a B wave" was ever seen. The possibility that these intermediate fibers from the several contributory sensory roots might on redistribution be so grouped in the saphenous as to produce the B wave did not occur to us. On the other hand, upon stimulation of an appropriate gray ramus a wave occupying the B position occasionally appeared in the peripheral nerve, and the search, therefore, was concluded with the statement that "whatever the meaning of this experience may be, our experiments have disclosed no other source of B fibers than the gray rami." While these experiments do show that in the cat axon potentials contributing to the B elevation may occasionally reach peripheral nerves via the gray rami they do not, we now feel, prove that sensory root fibers contribute nothing to B.

SUMMARY

Individual axons of small nerves of the frog have been identified by their action potentials through the cathode ray oscillograph after amplification up to 2,200,000 times and their properties determined.

To brief threshold shocks an axon responds with a latency that shifts spontaneously from a maximum of about 0.5σ in the case of the fastest fibers to over 2.4σ in slower fibers. The shift diminishes as shock strength increases and the response is stabilized at a latency, plus utilization period, of 0.06 to 0.1σ. A greater shift is observed in the case of fibers stimulated by a threshold constant current.

The irritability of axons also varies spontaneously through a narrow range; presumably this and the latency variations are an expression of spontaneous alterations in reactivity.

The characteristics studied are referred to conduction rate, as a base. Axon branching, examples of which are given, may, when unrecognized, lead to erroneous correlations.

The thresholds of individual axons, measured by induction shocks and condenser charges, increase as conduction rate decreases, along smooth curves of the hyperbolic type. There may be rather wide random variations.

The chronaxies, as conduction rate diminishes, fall uniformly from 0.3σ

to about 0.2σ at conduction rates of about 10 m.p.s., and then rapidly increase. The curve differs wholly from other irritability curves.

The curves expressing the relation of current strength to current duration for individual axons form a continuous series, the slower the fiber the further from the axis is its curve. The curves are not all quite identical in form.

The summation interval of a fiber fluctuates somewhat, but in different fibers shortens as the conduction rate slows, down to 2 m.p.s., and then lengthens rapidly. The lengthening is regarded as an effect of the high potential needed to stimulate the fibers of low irritability. The summation interval of nerve does not seem to be a measure of the speed with which the excitatory process subsides.

The determination of the relative recovery rates of axons after a response is complicated by the action of the second (testing) shock when, as in the case of the low irritability fibers, it must be made inordinately strong. It then delays the recovery process, even when delivered during the refractory period, with the result that in fibers of low irritability the absolutely refractory period, as measured, is as long as the relatively refractory period. The plotted recovery curves of individual axons form a continuous series, the slower the conduction rate of the fiber the further from the axes is its curve. The recovery curves are not all alike in form.

The recorded amplitude of the potential of axons diminishes as their conduction rates. The relation seems to be linear, but the variations are wide.

The time to maximum of the spike increases as conduction rate slows, the plotted points falling rather widely around a curve of the hyperbolic type. The difficulties in the way of correlating the above two values are considered, and some exceptional configurations of axon spikes are described.

It is pointed out that if the relation of the recorded spike height to conduction is linear the conduction rate in a fiber, granting certain assumptions, must vary as the square of its diameter.

By the measures that have been applied the properties of nerve fibers have been found to vary in a perfectly continuous manner from one end of the conduction rate range to the other. If there are fiber types they cannot be differentiated by their axon potentials.

Consideration is given to the bearing of these results on the interpretation of the significance of the elevations appearing on the conducted action potentials of nerves.

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THE CONFIGURATION OF AXON AND "SIMPLE" NERVE ACTION POTENTIALS

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The exact configuration of the electrical variation accompanying the activity of living cells is a matter of considerable interest and nerve is a particularly favorable structure from which to derive this information. Some of the difficulties that have to be overcome when using nerve for this purpose have been considered fully by Bishop (1927). These are *1*, differences in the propagation rates of the component fibers, and *2*, the spread of potential ahead of the active process. A third is the danger of indirect leads from active through inactive regions. All three of these difficulties could be avoided if it were possible to lead the action potential directly from the stimulated locus of a very thin nerve. Erlanger and Gasser in 1924 obtained records ostensibly from the stimulated locus but because of the artifacts produced by the stimulus, an induction shock, were unable to derive the exact form of the start of the response. Bishop (1927) then devised a method of balancing the shock artifact and obtained similar records from which the escape was largely, though not completely, suppressed, indicating that the rising phase of the unconducted submaximal α potential describes a very asymmetrical sigmoid.

At the time these studies were made it was believed that the potentials of the A axons all have the same temporal configuration and develop, in response to a short induction shock, after the same brief (ca 0.06 σ) utilization period (Erlanger, Gasser and Bishop, 1924). These conclusions, however, have been rendered untenable by the demonstration *1*, of a definite latency in the response of different fibers to stimulation, which increases with fiber threshold, and *2*, that the A spikes are not all alike in duration, but lengthen slightly as their thresholds increase. Submaximal action potentials led from the stimulated locus, recent observations indicate, are probably built up of spikes of fibers responding to the shock after intervals ranging from something less than 0.1 σ up to 0.3 σ and longer. They, therefore, are not all in phase and even if the axon potentials were all alike the shape of the rising phase of the summed potentials would depend upon their temporal spacing.

That the submaximal α action potential taken from the site of stimulation does not reproduce the temporal configuration of the axon spike is readily demonstrable by comparing the two obtained from one and the same preparation. In a typical experiment at 16°C. the time to maximum of the most irritable conducted axon spike was 0.47 σ (fig. 1). Under the same conditions a submaximal α spike recorded at the stimulated point and starting after 0.02 σ, had a time to maximum, measured from the beginning of the shock, of 0.94 σ. Since it has been shown (Blair and Erlanger, 1933) that the time to maximum of a late α axon spike is only a few per cent longer than that of the most irritable one, the disagreement between the times to maximum of these two deflections cannot be attributed to stimulation of fibers with slower spikes by the slight increase in shock strength needed to give the submaximal response, and must be attributed to temporal dispersion of the summed axon responses. Such dispersion can only result from a difference in utilization periods or in latent periods of the contributing axons. As the duration of the break shock from the air-cored coil employed is of the order of 0.03 σ, difference in utilization periods can account for no significant part of the dispersion. In this experiment, therefore, α fibers responded with latencies ranging from less than 0.2 σ up to 0.5 σ or more. The conducted axon spikes, as explained in the previous publication (Blair and Erlanger, 1933), are probably considerably prolonged by the spread of the axon potential proper along the nerve. Despite the presence of this foot, they are in all cases shorter by at least 30 per cent than the multifibered response at the stimulated locus.

The conducted axon spike, it has been shown (Blair and Erlanger, 1933), is deformed by the spread of potential and relatively more in large than in small nerves. It is definitely sigmoid in shape (fig. 3, *A*). In order to minimize this source of deformation the attempt was made to lead from the excited spot the axon potential initiated by a brief threshold induction shock using, to balance the escape, the modification of the method of Bishop described in the above-mentioned paper, but with the high amplification needed it was not possible to prevent deformation of the start of the axon potential by the shock escape.

Fig. 1. Comparison of duration of conducted axon spikes and of unconducted "simple" potential. A, B and C, spikes, probably of different axons; crest times average 0.47σ. D, submaximal α potential from the same nerve recorded at the locus of stimulation, with reduced amplification. E, shock escape, just below threshold.

Fig. 2. Axon spike from plantaris elicited by a threshold constant current, and recorded at the locus of stimulation on a base line consisting of a deflection during which the axon failed, through a spontaneous change in irritability, to respond to the constant current. The relation of the deflection to the coördinate system of the tube is indicated by the black line. 6/29/33. 29.7°C.

Fig. 4. A submaximal A potential from a nerve containing relatively few fibers; conduction 50 mm. 3/15/32. 15°C.

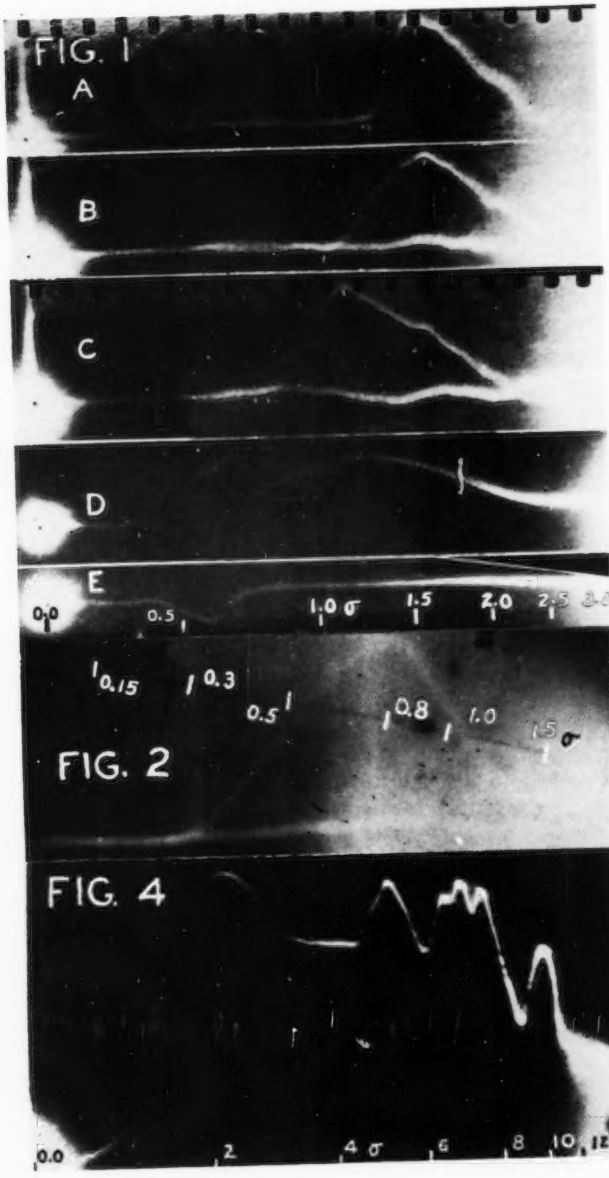
FIG. 1
A
B
C
D
E
0.0
0.5
1.0 σ
1.5
2.0
2.5
0.15
0.3
0.5
0.8
1.0
1.5 σ
FIG. 2
FIG. 4
0.0
2
4 σ
6
8
10
12σ

A threshold constant current, which was permitted to flow during the recording period, was then substituted for the induction shock. A long interval (utilization period) then elapses between the make of the current and the start of the action potential, an interval that is long enough to permit the transient disturbance associated with the make to disappear, and the action potential then records without the initial distortion (fig. 2). This method has the added advantage that at threshold the spontaneous variations in the nerve's irritability result in occasional failures of the nerve to fire off. One thus records under the action potential a perfectly valid base line. The responding point on the nerve, to be sure, is being polarized cathodally during the period of the record, but there are reasons for believing that threshold cathodal polarization does not affect the configuration of initial responses (Bishop and Erlanger, 1926).

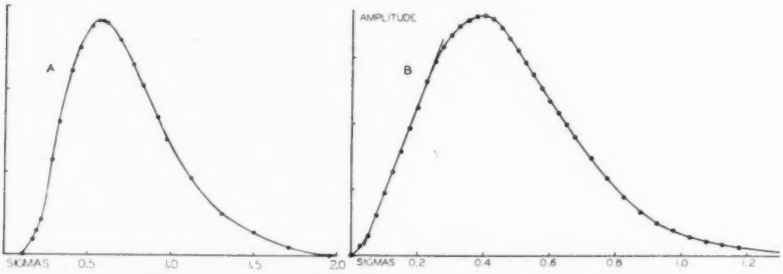

Fig. 3. *A*. Conducted axon spike from plantaris for comparison with *B*.
B. The axon spike of figure 2 transferred from semilogarithmic to Cartesian coördinates. Altitude measured from its own base line.

It has not been emphasized in the past, however, that records thus obtained do not come exactly from the stimulated locus. Although the stimulating cathode and the ground are one, the lead has some width and stimulation must occur at the edge proximal to the anode whereas the potential is led from the edge proximal to the grid. The lead electrode is about 1 mm. in width. Therefore, if the impulses recorded in these experiments traveled at the rate of 20 m.p.s., potential spreading instantly from the excited spot would have begun to record some 0.05 σ ahead of the action potential proper.

Such a record, transferred to linear, rectangular coördinates, is shown in figure 3, *B*. It differs very decidedly in appearance from a conducted axon potential. For more than two-thirds of the height the ascending limb is a straight line. The foot is uniquely low and less than 0.05 σ in duration. It seems justifiable, therefore, to conclude not only that the local process starts at full speed but also that it suffers no obvious retardation until (in this case) it acquires over 80 per cent of its full potential.

On the basis of this picture one is justified in going further than Bishop, who inferred that "the axon response. . . . . assumes the character of an explosive reaction," and conclude that the axon response is explosive.

On the configuration of "simple" action potentials. As ordinarily recorded, a submaximal α action potential, or any other "simple" potential from a large nerve, always has a perfectly smooth contour. It may be of some interest to observe how this smoothness is derived from the irregular pictures that one obtains when only a few axons respond.

If, with very high amplification, the just threshold response of a large nerve is observed, it is seen that practically every one of the few responding axons shows random variations both in shock-spike time and in irritability; the result is a wholly unpredictable picture as shown in figure 2 of Blair and Erlanger (1933). In the case of such large nerves the shunting mass distorts the spikes so that any single axon spike starts with a long low foot which is terminated by a very steep rise to maximum. On a logarithmic time line, this sudden change in rate produces a sharp angle on the rising phase. Because the axon responses, due to shunting, are low the foot is not visible in these records and the axon spikes can only be measured from angle to crest. The values thus obtained are, therefore, usually much smaller than the over all rising phases of the conducted axon spikes here reproduced. A crest time of 0.2 σ is not unusual.

When the conditions are such that a single axon spike represents a significant fraction of the total nerve potential, the manner of growth of the multifiber response can be followed as more and more fibers are brought into the picture by gradually increasing the strength of stimulation. A single axon spike first appears, infrequently and with a highly variable shock-spike interval. Then the responses become more frequent and the shock-spike interval shortens and varies less. Eventually the axon responds to every stimulus with a fixed, short shock-spike interval. Less irritable units go through a like sequence, but because of their longer minimum latencies their stabilized shock-spike intervals are longer. They exhibit greater play in irritability and latency than did the more irritable fibers, but this play cannot be followed so clearly because the spikes are superimposed on the summed potentials of the more irritable fibers. In a large nerve the high irritability fibers, stimulated supramaximally by a shock which produces, say, a half maximal α response, give to the "simple" potential its very short absolute latency and its characteristic, smooth, steep rising phase, and such variations as may be occurring in the responses of the fibers of low irritability stimulated at threshold are smoothed over by the randomness of the variations of the large numbers involved.

Any procedure evoking responses of only a few fibers will result in an irregular figure (see fig. 4); and if any of the fibers are being stimulated just at threshold, the successive pictures will vary in configuration, provided the amplification be sufficient to show the increments from single axons.

SUMMARY

It is shown that the temporal configuration of an α action potential initiated by an induction shock and led from the stimulated point differs materially from that of an axon potential similarly obtained but by a method circumventing the distortion of the start by the stimulus artifact. The axon potential thus obtained exhibits *1*, a low short foot which can be accounted for by the width of the common electrode separating the stimulated point from the lead, terminated *2*, by a rise that is linear to within 80 per cent of the crest. It is concluded that the action potential mounts linearly from its inception.

The configuration of the "simple" action potential is considered in the light of these findings and of the variations in the latency of response, and in the irritability, of axons.

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FACTORS WHICH DETERMINE RENAL WEIGHT

XV. The Relation Between the Vitamin B (Old Terminology) and Protein Intakes

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Reader and Drummond (1) claimed that renal hypertrophy does not occur in rats maintained on a high protein régime if the supplements of vitamin B (used here and throughout this paper is the old term including vitamins B, G and undifferentiated B as found in yeast and yeast extract) are sufficiently increased. Hartwell (2) states that yeast or its heat stable moiety prevents enlargement of the kidneys. Francis, Smith and Moise (3) on the other hand conclude that the increase in the size of the kidneys which follows the administration of a high protein diet is neither lessened nor prevented by the use of any amount of vitamin B or combination of its component parts. Experiments upon the point in question are considered here.

The methods have been described (6), (7). In half the cages coprophagy was prevented by screens. As might be expected when there was no true vitamin deficiency the results were the same as in the group allowed access to their feces. The composition of the diets comprise table 1.

Four groups each made up of 10 male and 10 female rats were fed from 40 to 70 days of age. Two groups received normal casein diets. Another pair were given a high casein diet. One of each pair (1 and 3) had the usual vitamin B intake and the others (2 and 4) a high vitamin B intake in the form of yeast extract. The differences in the averages (table 2) were found to be statistically significant. The results show definitely that the high protein diet group with the high vitamin B intake (group 4 with the intake of vitamin B in the same relation to the casein as in the normal diet of group 1) had smaller kidneys than the high casein low vitamin B group (group 3). This is in spite of the fact (table 2) that this group ingested appreciably more protein than group 3. Although the yeast extract greatly lessened the increase in renal weight which might be expected from a high protein diet it did not entirely prevent the renal hypertrophy.

Formulae have been presented elsewhere in which the relationship between the protein intake as casein and the renal weight has been expressed:

for male rats (7),

$$\text{Renal weight} = (\text{Protein intake} + 2.75) \div 0.0183$$

for female rats (8),

$$\text{Renal weight} = (\text{Protein intake} + 2.2875) \div 0.0183$$

If we calculate the expected kidney weight from these formulae (table 2) we find that instead of the expected 60 per cent renal hypertrophy for group 4 it amounted to only 17 per cent.

TABLE 1

	DIET 1	DIET 2	DIET 3	DIET 4
Casein*	20	20	80	80
Cornstarch†	66	60	6	0
Salt mixture‡	4	4	4	4
Cod-liver oil	8	8	8	8
Yeast extract§	2	8	2	8

* Crude commercial casein.

† Raw cornstarch.

‡ Osborne and Mendel (4) salt mixture.

§ Vitamin B concentrate according to Osborne and Wakeman (5)—Harris Laboratories, Tuckahoe, New York.

TABLE 2

GROUP	NUMBER OF RATS	BODY WEIGHT	BODY LENGTH	HEART WEIGHT	KIDNEY WEIGHT PER 100					INTAKE PER 100 SQ. CM. BODY SURFACE PER DAY		KIDNEY WEIGHT CALCULATED FROM PROTEIN INTAKE IN RELATION TO BODY SURFACE	DEVIATION OF CALCULATED FROM OBSERVED KIDNEY WEIGHT	
					mm. body length	mgm. heart weight	sq. cm. body surface						Absolute	Per cent
							Mean	P. E.	Coef. Var.	Food	Protein			
		gm.	mm.	mgm.	mgm.	mgm.	mgm.	mgm.	per cent	gm.	gm.	mgm.	mgm.	
1	20	166	188	522	308	111	170	2.7	11.2	2.66	0.47	164	−6	−3.5
2	20	164	187	538	294	103	163	1.8	7.1	2.74	0.56	168	+5	+3.0
3	20	132	180	474	359	137	220	2.0	5.9	3.05	2.06	251	+31	+14.1
4	20	133	180	470	328	125	198	1.5	4.9	3.38	2.28	263	+65	+32.9

We have pointed out before (7) that as the protein concentration in the diet is increased above 30 per cent of the calories, growth becomes progressively poorer. It seemed possible that this might not have been the case if a constant ratio between the protein and yeast extract contents of the diet was maintained as Reader and Drummond (1) have found neces-

sary. This hypothesis is not borne out by the present experiments. At the beginning of feeding the average body weights of all four groups were practically identical. The weights at death indicate that only the protein concentration of the diet and not the amount of yeast extract determined the rate of growth.

SUMMARY

The increase in the weight of the kidneys of the albino rat produced by a high protein (casein) diet is greatly lessened but not entirely prevented by maintaining the same vitamin B (old terminology, represented here by yeast extract): Casein ratio in the low and high protein diets.

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THE NATURE OF THE ELECTRICAL FIELD AROUND THE HEART

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In a series of experiments dealing with the distribution of potential around the tortoise heart, in situ and isolated in conducting media of several different shapes, it became evident that the potentials at points in the body distant from the heart were larger than those found in a homogeneous conductor of the shape and volume of the animal. A similar situation appeared to exist in the mammal. If the body represented a uniform electrical field approximating a sphere, as has been generally assumed since the analysis of Einthoven, Fahr and de Waart (1),[1] the potentials recorded in the electrocardiogram by the standard leads would be very small as compared to the potentials at the heart, and the difference between the potentials at the two points would be greater than that actually found. The nature of the conducting field determines not only the magnitude of potentials present at points within it, but also to a certain extent the form of the resulting potential curve arising from the summation of the individual action potentials. Any attempt to determine the nature of the electrical processes originating in the muscle and to correlate these with the electrocardiogram must therefore take into account the character of the field in which summation of the potentials and conduction to the periphery occurs.

Two series of experiments were carried out in the study of this problem. The first comprised a determination of the potentials at the peak of the R wave at different distances from the tortoise heart and a comparison between those found with the heart in situ and with the heart isolated in conducting fields of different form. The second series, performed on dogs and rabbits, was concerned with the electrical conductance of various paths in the tissues.

Apparatus. The experiments on the tortoise heart necessitated the

[1] The Einthoven triangle represents a plane through this sphere, in which the vector representing at any instant the direction and magnitude of the electrical force, is the projection in this plane of the three dimensional summation of all electrical forces present. It is well recognized that this analysis, although of considerable theoretical and practical interest, is to be regarded as an approximation only (2).

accurate determination of potentials as small as two or three microvolts. A string galvanometer was employed as the recording instrument, because it was possible to obtain sufficient sensitivity with it by providing only two stages of vacuum tube amplification. Since direct coupling is feasible when only two stages are employed, this type of coupling could be used and accuracy of potential measurements secured at all frequencies within the frequency range of the galvanometer itself. The circuit diagram of the amplifier is given in figure 1. Connection to the galvanometer is through

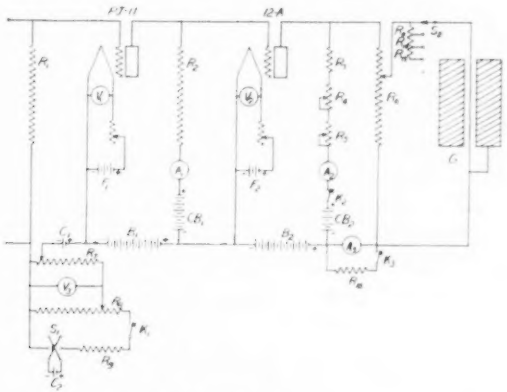

Fig. 1. Two stage direct coupled amplifier.

Constants of circuit:

Resistors in ohms. Carbon, R_1, 500,000, R_{12}, 1,000,000, R_{12}, 100,000, R_{11}, 10,000. Wire wound, non-inductive, R_2, 500,000. Wire wound, R_3, R_4, 10,000, R_5, 400, R_6, 18,000, R_8, 200, R_9, 75, R_{10}, 1.122. R_7, wire wound non-inductive, total 500, in steps of 1, 1, 3, 5, 10, 30, 50, 100, 300.

Voltmeters. V_1 V_2, 0–5 volts, V_3, 25–0–25 millivolts.

Milliammeters. A_1, 0–2, A_2, 0–15, A_3, 5–0–5 milliamperes and 100–0–100 microamperes.

Keys and switches. K_1, calibration, K_2, compensation circuit, K_3, to change range of A_3, S_1, calibration commutator, S_2, protective resistance.

Batteries, in volts. C_1, C_2, 1.5, F_1, F_2, 6, B_1, B_2, CB_1, CB_2, each 112.5.

Tubes. General Electric P.J.-11, R.C.A. 12-A.

Shielding. Amplifier in copper case. Leads to preparation in shielded cable.

a balanced circuit by means of which the steady plate current of the second tube is compensated. The extent of voltage amplification can be varied from unity to approximately two hundred and fifty, and a maximum sensitivity of 1200 mm. per millivolt obtained with the tension of the galvanometer fiber adjusted to the point of critical damping. The use of the amplifier in connection with the string galvanometer was necessary not only to obtain sufficient sensitivity, but also to allow potential rather than

current measurements to be made. The string galvanometer is essentially a current operated instrument, markedly affected by the resistance in the circuit. By using an amplifier, the input resistance of which is of the order of a million ohms, influences due to changes in resistance between the electrodes in contact with the conducting field become negligible. The high input resistance also avoids the distortion of the field which occurs when a circuit of relatively low resistance is introduced.

Measurements of the electrical impedance over different conducting paths in the mammalian experiments were made with an alternating current

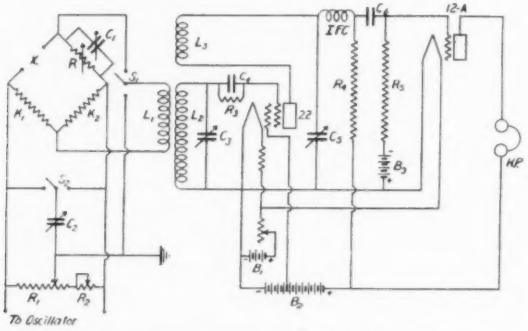

Fig. 2. Alternating current bridge, detector and amplifier.

Constants of circuit:

Resistors, in ohms. K_1 K_2, carbon, 10,000, R, non-inductive decade 10,000 (Leeds and Northrup 4745), R_1, 10,000, R_2, 400, R_3, R_4, R_5, each 500,000.

Capicitances in microfarads. C_1, 0.005 (General Radio, 246-P), C_2, 0.00035, C_3, 0.005, C_4, 0.001, C_5, 0.0015, C_6, 0.25.

Inductances. L_1, 200 turns, scramble wound, L_2 and L_3, honeycomb wound, 1000 turns. I.F.C., 500 turns, scramble wound.

Batteries, in volts. B_1, 6, B_2, 135, B_3, 4.5.

Tubes. R.C.A. 22 in space charge connection. R.C.A. 12-A.

Shielding. The oscillator and its supply batteries are in one shielded compartment, the bridge proper in a second, the detector and amplifier with their supply in a third. Leads between these shields and to the preparation run in shielded cables. The component parts of the bridge are shielded separately.

Tuning range of detector—10,000 to 60,000 cycles.

bridge. Current of 40,000 cycles was obtained from a vacuum tube oscillator (General Radio, type 377-B). The harmonic content of this oscillator is less than three per cent. The output from the oscillator was applied to the animal through electrodes of block tin connected to the shaved skin of the right fore and left hind legs. The bridge used for impedance measurement was designed with care to avoid the introduction of inductance or unknown capacitance into the circuit, and in general follows modern practice. The balancing arm is a Kohlrausch network, consisting of a

capacitance in parallel with a resistance. A modified Wagner ground is employed to balance out stray capacities. The null point of the bridge is very sharp, readings being made with an accuracy of less than an ohm within the range of resistances encountered in these experiments. The circuit diagram is given in figure 2.

The fall of potential around the tortoise heart. In these experiments the plastron was removed, two non-polarizable electrodes placed along the same axis, at equal distances from the heart but on opposite sides of it, and a record made of the deflections of the galvanometer string. The electrodes were now moved to a new position along the same axis and a record made as before. In this way some eight or ten records were obtained representing the potential curve during the course of the heart beat at different distances from the heart, the body tissues representing the conducting field. In each case the amplification was adjusted to give sufficiently large deflections for accurate measurement, and calibration of sensitivity was made on the record. The magnitude of each of the R waves was now measured, expressed in terms of total potential by reference to the calibration curve, and the values plotted against distance from the center of the heart. The curves so obtained were of hyperbolic form, showing at first a very rapid fall, which became more gradual as the distance from the heart was increased. The vessels entering and leaving the heart were now ligated and cut, and the heart removed and placed in the center of a circular pad of filter paper wet with Ringer's solution. Similar records of potential at different distances along the same axis as before supplied the data for a new curve to be compared with the one made with the heart in situ. As a final procedure the tissues of the tortoise were removed, the shell filled with an equivalent volume of Ringer's solution, the heart placed as near to its original position within the shell as possible, and potentials recorded along the same axis as used in the other determinations. In a number of experiments, determinations were also made with the heart suspended in the center of a glass globe, 40 cm. in diameter, filled with Ringer's solution.

The curves obtained under these different conditions were always similar in form but differed in the rapidity with which the potential fell as the distance from the heart increased. The rapidity of fall was somewhat less in a circular plane conducting field than in the case of the heart in situ, but the two curves were not widely different. In all cases, however, the curves obtained with a homogeneous volume conductor, either the sphere or the shell filled with Ringer's solution, showed a very much greater rate of falling off of potential, and differed widely from the other two curves. In the homogeneous volume conductor the potentials at moderate distances from the heart were not more than one-tenth those found with the heart in situ. Figure 3 is a graph of the potentials under the conditions described, and is typical of a large number of experiments performed.

Before these results can be interpreted as representing differences due to the shape of the conducting field, it is necessary to show that the electrical conductance of the media does not differ greatly under the different conditions described, since this alone would affect the slope of the curve. In the

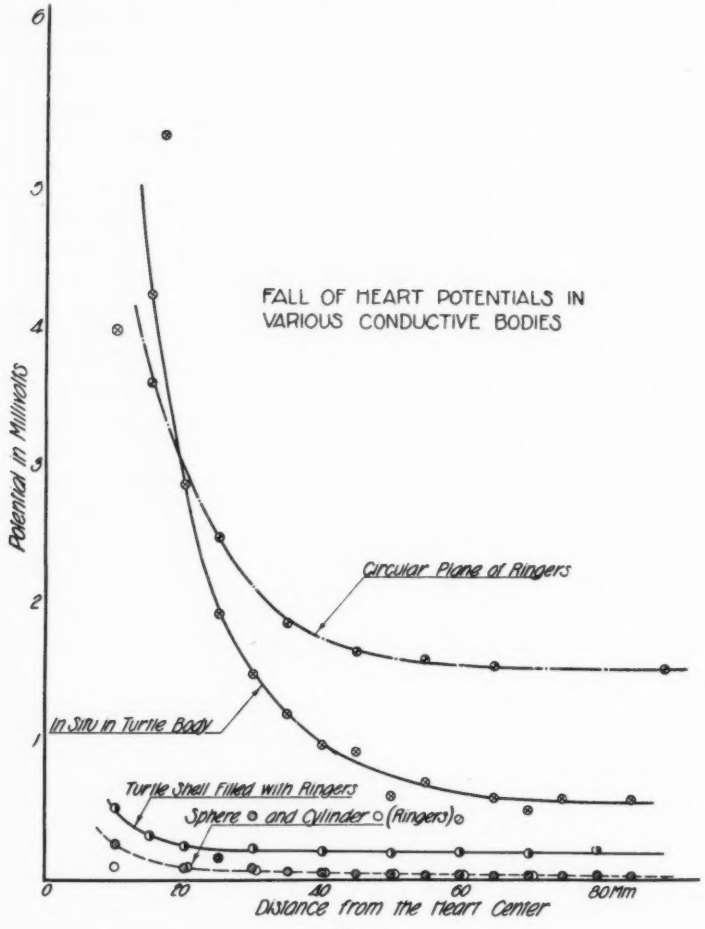

Fig. 3

mathematical formulae defining the potential in a disc or volume (spherical) conductor, arising from an electrical source and sink at the center, specific conductance or conductivity enters as a factor in the denominator (3). Increase in conductivity of the medium hence reduces the potential

at any point in the medium, all other factors remaining constant. If the conductance of Ringer's solution were ten times as great as that of the tissues of the tortoise, the differences in potential found between the heart in situ and in a volume of Ringer's solution could be explained entirely on this difference in conductance. Direct determination shows however that no such differences in conductance exists. This determination was carried out in the following manner. The plastron of a tortoise was removed and non-polarizable electrodes placed in contact with the tissues in a certain fixed position. The electrical impedance in the circuit was determined by means of the alternating current bridge, using a frequency of 4×10^4 cycles. The tissues of the tortoise were now removed and an equivalent volume of Ringer's solution poured into the shell. The electrodes were placed in contact with the solution at the same positions relative to the shell as before, and the impedance again determined. The difference in impedance in the two cases differed by less than six per cent, being slightly higher with the tissue than with the Ringer's solution. The difference in electrical conductivity of the mixed tissues and the Ringer's solution by which they were replaced in our experiments is thus negligible.

It is thus clear that the differences between the potentials found with the heart in situ, isolated in a plane conducting medium, and in a volume conductor are to be referred to the shape of the conductor. The fact that the first two do not differ greatly while the last departs widely, leads to the conclusion that the body tissues of the tortoise represent a conducting medium approximating a plane rather than a homogeneous volume conductor the shape of the body. Since all tissues conduct an electrical current, this implies that of the various tissue conducting paths, one or more are better conductors than the remainder, and that the more highly conducting paths approximate a plane in shape.

Electrical conductance in the mammal. The type of electrical circuit represented by tissues is a complex one and not clearly understood. Little advance in knowledge was made until the application of modern methods of resistance measurements employing the alternating current bridge. Gildemeister (4) showed that in order to obtain balance in a bridge circuit in connection with resistance measurements of tissues, a capacitance was necessary, an observation previously made by Kohlrausch in the case of electrolytic solutions. The tissues hence manifest capacitative reactance as well as direct current resistance and the former varies with the frequency of the applied current. The nature of the capacitance is also not entirely clear. It is probably in part due to the presence of membranes of low ionic permeability acting as areas of high resistance, thus approaching a dielectric in electrical characteristics, but forming "leaky" electrostatic condensers (5). There is also evidence for the presence of "polarization capacity," ionic polarization at interfaces due to differences in ionic perme-

ability characteristic of membranes. This appears to be especially important in the skin (6). It is markedly altered by the passage of direct current or of oscillating currents of low frequency. The reactance of the skin may be greatly reduced by the application of a constant current, due, it is believed, to membrane depolarization. When the current is withdrawn an e.m.f. of considerable magnitude and with opposite polarity to that of the applied current is present for a brief period.

The impedance of the body or body tissues thus varies with the frequency of the alternating current used to measure it. It decreases with increasing frequency and tends to approach a constant value above one million cycles. Using such high frequencies, one fails to evaluate the capacitance factor and such resistance as may be in parallel with it. On the other hand, if a low frequency is employed, polarization changes occur which alter the conditions existing in the tissue through which the current flows. The hypothesis, that electrical stimulation of excitable tissue depends upon a sufficient duration (as well as intensity), of the unidirectional phase of the applied current to produce a certain grade of polarization change, is supported by many experimental observations. The potential required to stimulate is thus a function of the frequency. It rises at first slowly and then very rapidly as the frequency is increased above thirty or forty thousand cycles. That changes in resistance and capacity occur during activity has been shown in voluntary muscle (7) and in heart muscle (8).

The complete solution of the problem of electrical conduction in the body would involve the determination of the specific resistance (resistance per unit length and unit cross-sectional area) for each kind of tissue, and a knowledge of the volume distribution of each particular tissue. Approximate specific resistances of certain tissues and of blood serum, measured at high frequency (10^6 cycles) in order to eliminate changes due to isolation and death, have been obtained (9, 10). These determinations would indicate that for gross tissue, the lowest specific resistance is present in the blood (less than 100 ohms), approximated closely by that of skeletal muscle (110 ohms). The specific resistance of skin is somewhat higher (289 ohms) and that for bone and fat much greater (1800 and 2180 ohms respectively). Highly vascular organs, such as the kidney, heart, liver and spleen showed specific resistances varying between 126 and 298 ohms. It is to be noted that with the high frequency employed the capacitance reactance is negligible, and the total impedance is less than that which would be found at low frequencies.

Even were it possible to obtain accurate figures for total specific resistance for different tissues and organs in the body as a whole, the multiplicity of paths of conduction separated in part by tissues of varying degrees of relatively high resistance, would present a problem apparently impossible

of satisfactory theoretical solution. The orientation of the tissue, especially muscle, in any given path is likewise of importance. Thus the transverse resistance in living muscle has been found to be seven times as great as the longitudinal resistance (11). This difference appears to be associated with reactance as well as resistance in the transverse direction, due to the membranes (12), and disappears after death of the muscle. Variations from time to time in the relative blood content in different regions may cause variations in the resistance of certain paths in the living animal, and relatively slight variations in extracellular fluid content probably cause marked alterations.

It seems well established that resistance in the tissues exists in series as well as in parallel with the capacitance Remington (13) and McClendon (14) have demonstrated this in an ingenious manner and obtained an estimate of the magnitude of the series resistance, by comparing the total impedances at one thousand and one million cycles respectively. At the latter frequency the capacitative reactance in the tissues approaches zero and short circuits the resistance in parallel with it, leaving the series resistance as the total impedance. The tissue circuit may be even more complicated than this. Frick and Morse (10) determined the impedance of blood corpuscle suspensions at eight separate frequencies between 8.7×10^4 and 4.52×10^6 cycles, and found that the experimental values approximated closely the theoretical values for a circuit consisting of a resistance in series with a condenser, both shunted by a parallel resistance.

In attempting to compare resistance and capacity in various tissues or body pathways, one is confronted by certain considerations which must determine the details of the method employed. The more important of these are the following: *a*. The frequency should be higher than that which will stimulate tissue at the voltage and current density used. It is possible that this will not prevent some alteration in the tissue due to passage of the current, but it will at least avoid the marked alteration of ionic distribution and membrane polarization associated with stimulation. *b*. The frequency should not be so high that it fails to give some information of the capacitative reactance, which is an integral part of the total impedance at low frequencies, and which may differ over different pathways. The ideal frequency from this standpoint would be the same as the frequency of the current, the distribution of which in the body is the object of study, specifically in the present instance, the frequency of the summated action currents of the heart.

The intrinsic difficulties of the problem of electrical conducting paths in the animal are evident from the preceding discussion and would seem to justify a rather wide approximation to the exact quantitative solution. The data as presented in this paper should be so regarded. We have selected more or less arbitrarily certain conducting pathways and have

recorded resistance and capacity in a bridge the balancing arm of which is simpler than the path followed by the current in the tissues. Our results have to do only with the comparative impedance over these different paths at the arbitrary frequency employed.

Changes in impedance during ether anesthesia and after death. Since our determinations were made under one or the other of these conditions, it was necessary to run controls to determine the effect of these factors in our experiments. Changes in impedance, or of resistance or capacity alone, might conceivably result from *a*, drying of electrodes; *b*, fall in body temperature, or *c*, alterations in the tissues due to the anesthesia or following death.

While precautions were taken to reduce gradual drying of the electrodes, this could not be entirely prevented. Since a fall of temperature is associated with a rise of impedance in the case of solutions of electrolytes, it was to be expected that the fall of body temperature under anesthesia or after death would also induce a rise of impedance. This relationship has been shown to occur in experiments in which dead animals were placed in a controlled temperature chamber (9). The impedance to high frequency current (10^6 cycles) varied inversely as the temperature. That changes occur in the tissues after death altering tissue impedance independent of temperature changes, has also been shown by a number of workers (15, 16). Hemingway and Collins (5) found definite changes to low frequency, none to high, indicating that the process was one of altered membrane permeability rather than one of changes in ionic concentration of the cellular or intercellular contents (17, 18). In most of the work quoted there was found a fairly rapid rise of total impedance for the first few hours after death, succeeded by a slower fall to a point lower than the value at the time of death.

It was found under ether anesthesia and for several hours after death that there is a steady rise of impedance under the conditions of our experiments. This rise amounted on the average to about 18 ohms per hour and showed little variation in the different determinations. This correction has been applied to the data in the other experiments. If the impedance and body temperature are plotted, the relation between the two curves suggests that during anesthesia and for a certain period after death the rise in impedance is due to the accompanying fall of body temperature.[2] Later, other factors enter, associated perhaps with blood coagulation, alteration of membrane permeability and tissue autolysis.

The impedance and conductance of the body structures in the dog. Electrodes were attached to the right fore and left hind legs and connected to

[2] In the expression $K = ZT$ where Z is the impedance and T the temperature, K has nearly a constant value during anesthesia and for over an hour after death. Subsequent to this it begins to decrease in value.

the bridge for impedance measurements. The animals were placed under ether anesthesia, and a series of measurements made until the approximate rate of rise of impedance associated with the anesthesia was established. There was now carried through successive separation of body structures as follows, several determinations of impedance being made after each procedure. The skin and subcutaneous tissues were first cut through around the mid region of the abdomen. The retraction of the cut skin

TABLE 1

Impedance in ohms between the right fore and left hind legs over different conducting paths in the anesthetized dog

CONDUCTING PATH	IMPEDANCE IN OHMS*							
	1	2	3	4	5	6	7	Average
All paths	253	361	372	316	381	310	289	326
Skin and subcutaneous tissue	1,602	7,200	100,000	8,070	143,000	5,720	9,815	39,344
Abdominal viscera**	6,420	3,879	4,260	4,085	4,065	2,820	4,528	4,294
Abdominal aorta and vena cava	83,330	125,000	167,000	143,000	20,820	71,500	83,400	99,150
Muscles of anterior and lateral abdominal wall	3,665	14,300	5,500	2,780	6,810	5,000	4,810	6,124
Dorsal muscles and vertebral column	508	578	588	560	599	528	454	545
Spinal cord	1,085	1,758	1,804	1,566	1,970	1,701	1,400	1,612
Combination of last two paths	408	514	443	412	459	403	343	426

* Impedance (absolute value) was calculated from the bridge readings by the formula $Z = \frac{R}{\sqrt{1 + R^2C^2\omega^2}}$ where R is the resistance and C the capacity in the balancing arm, ω equals $2\pi f$, where f is the frequency, held constant in all determinations at 4×10^4 cycles. Impedances of the various paths were computed by the formula $\frac{1}{Z} = \frac{1}{Z_1} + \frac{1}{Z_2} + \ldots \frac{1}{Z_n}$ where $Z_1 Z_2 \ldots\ldots Z_n$ represent the impedances in the different paths and Z the total impedance.

** With the exception of the liver.

resulted in a circular belt of separation several centimeters wide. The abdomen was now opened by a midline incision and the abdominal and pelvic viscera, with the exception of the liver, removed. The abdominal aorta and vena cava were ligated and sectioned. The abdominal muscles were next severed, following along the line of the original skin incision, back to but not including the thick muscle mass, formed of the iliocostalis lumborum, longissimus, spinalis and semispinalis and multifidus muscles, surrounding the spinal column. These latter muscles and the spinal column

were now severed, leaving the spinal cord as the sole connecting path. This was finally severed as a control for possible current leakage through the table or supports.

Following each procedure there was a rise of impedance, the extent of which depended upon the importance of the severed path in electrical conduction. From the data so obtained, corrected for the rise in impedance associated with the anesthesia alone, the impedance and conductance over the individual paths were calculated. These values are given in seven experiments of this kind in tables 1 and 2.

TABLE 2

Percentage of total body conductance of different paths in the anesthetized dog

PATHS	CONDUCTANCE IN PER CENT OF TOTAL*							
	1	2	3	4	5	6	7	Average
Skin and subcutaneous tissue	15.76	5.01	0.37	3.92	0.27	5.43	2.95	4.81
Abdominal viscera**	3.90	9.20	8.74	7.74	9.36	11.00	6.38	8.05
Abdominal aorta and vena cava	0.34	0.29	0.22	0.22	1.83	0.43	0.35	0.52
Muscles of anterior and lateral abdominal wall	6.90	2.50	6.77	11.37	5.59	6.19	6.02	6.48
Dorsal muscles and vertebral column	49.90	62.50	63.35	56.55	63.62	58.70	63.96	59.80
Spinal cord	23.20	20.50	20.55	20.20	19.33	18.25		20.34
Combination of last two paths	73.10	83.00	83.90	76.75	82.95	76.95	84.30	80.14

* Conductance over the various paths was computed from $\frac{1}{Z}$ and is expressed in percentage of the total conductance of the body, taken as 100 per cent. It should be noted that conductance in the sense used here has the same meaning as the absolute value of admittance in alternating current theory.

** With the exception of the liver.

The impedance of the skin and subcutaneous tissue shows considerable variation in different animals, but in general is high. These differences appeared to be related to the degree of vascularity. In most cases no effort was made to distinguish between skin and subcutaneous tissue, but in a few cases this was done and skin impedance was found to account for about two-thirds, subcutaneous impedance for one-third of the total. Percentage conductance through the skin and subcutaneous tissue amounts on the average to less than five per cent of the total conductance. Impedance through the abdominal and pelvic viscera is much more uniform in the different determinations and less (in all but one experiment) than that through the skin and subcutaneous tissue. The viscera however

account on the average for only eight per cent of the total conductance and it is perhaps surprising that so large a mass of tissue offers so poor a conducting path. On the other hand, it is well known that a large part of tissue impedance is due to membranes, and in this path many dense membranes intervene.

Section of the aorta and vena cava caused little change in impedance in the total circuit. While our results point, as will be evident later, to the general vascular path as an important and perhaps the most important single electrical conducting path in the body, this refers rather to the more peripheral portions of the vascular system, where the total cross-sectional area of the conducting path is large.

The importance of the anterior and lateral muscles of the abdominal wall as a conducting path is also not great, presumably due to their comparative thinness. The most consistent and marked change in impedance occurred on sectioning the dorsal muscles and vertebral column. The resistance of this path is less than that of any other, accounting for nearly sixty per cent of the total conductance of the trunk. The dorsal muscles present a thick, highly vascular muscle mass. Efforts were made in a few experiments to distinguish between the muscles and vertebral column, but the difficulty of clearing the latter of muscular and vascular connections was such that consistent results were not obtained. It was evident, however, that severing the bone of the spinal column itself was of insignificant effect as compared with the clean separation of muscles and vessels thus obtained. Next to the dorsal muscles, the path of lowest impedance was that of the spinal cord. In the last row of the two tables the impedance and percentage conductance over the combined dorsal muscle and cord path is given. This path accounts for approximately four-fifths of the total conductance of the trunk. The electrical conducting path of the trunk thus is concentrated to a large extent over a relatively small part of the total volume.

The importance of the blood vascular system in electrical conduction. It is well known that much of the electrical impedance in tissues is due to the presence of membranes, associated probably with their low permeability to anions. It appears that the relatively high impedance of the path through the abdominal viscera may be due to the frequent interposition of membranes. These and other considerations suggest the blood vascular system as an important path of electrical conduction in the body, particularly the more peripheral portions, where extensive branching has occurred and where many parallel paths of conduction are present with few membranes interposed. According to determinations made on freshly killed rabbits (19), the resting muscles contain nearly a third of the total blood, while the amount in the skin and abdominal viscera (with the exception of the liver) is much less. The importance of the thick dorsal muscles as a

conducting path in the dog would thus suggest that this may be due in part to the relatively large mass of blood contained within them.

We have made an attempt to determine the importance of the vascular system as a conducting medium by replacement of the blood in the freshly

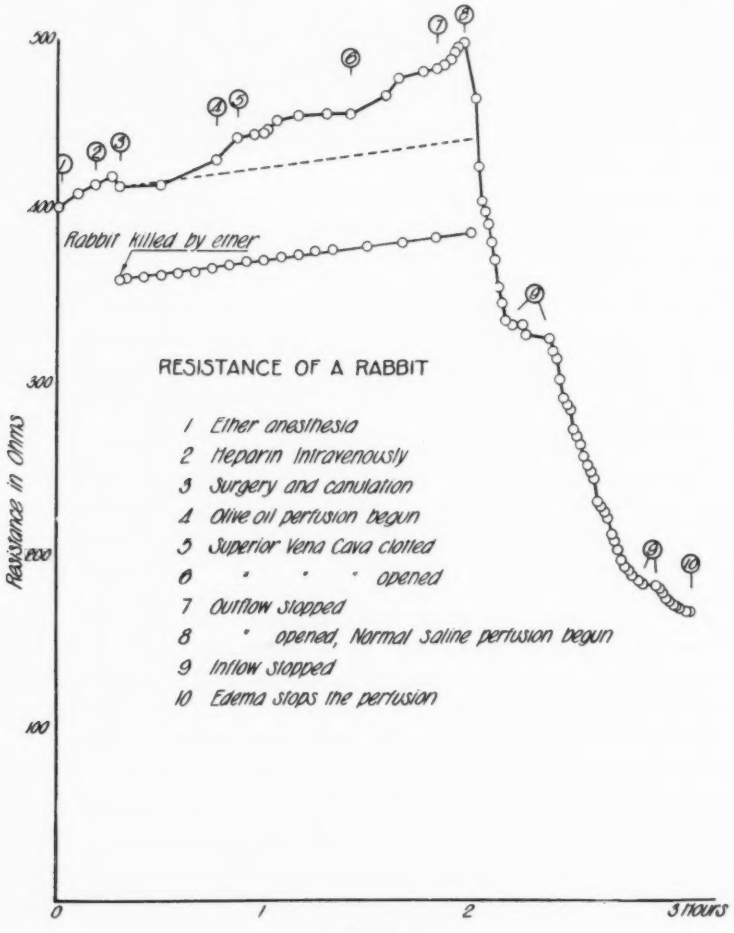

Fig. 4

killed animal by an electrically non-conducting oil. Four rabbits and two dogs were used. The animals were anesthetized, inflow and outflow cannulae placed in the thoracic aorta and vena cavae respectively, and oil forced into the vascular system under pressure. Nujol, paraffin oil, olive

oil and kerosene were tried. The attempt at blood replacement was only partially successful, examination in each case revealing that the oil failed to fill certain vascular regions even after long perfusion. Paths of lower flow resistance became established, other paths filling only partially. The increase in total body impedance after partial replacement of the blood by oil in the six experiments amounted to from 20 to 34 per cent of the initial impedance. These differences we believe to be due to differences in the extent of blood replacement, and that the higher figure represents more nearly the significance of the vascular system in electrical conduction. In the experiment showing the maximum rise of impedance after partial blood replacement, the resistance over the blood path was 2,040 ohms, over all other tissues 685 ohms. The blood, forming approximately 10 per cent of the total volume of the body, thus accounted for more than one-fourth of the total conductance. Figure 4 gives a record of a similar experiment showing the smallest change encountered, in which partial replacement of the blood by olive oil caused a rise from 405 to 500 ohms, an increase of 95 ohms. This increase is reduced to 80 ohms on applying the correction for rise of impedance following death.

The question as to the importance of blood as a conducting medium can also be studied by increasing the total blood volume by transfusion. It is known that most of the excess blood under these circumstances is contained in the peripheral vascular bed (20). A small dog, weighing 5.22 kilos, was connected to the bridge in the usual way, and its total blood volume increased by transfusion from a larger dog. The recipient was placed on a weighing balance, so that the amount of blood received could be estimated. Approximately 660 cc. blood were transfused in a period of 30 minutes. The impedance, initially 456 ohms, fell to 416 ohms. Applying a correction of 9 ohms, the approximate rise that would have otherwise occurred during this time, we have a total fall of 49 ohms, a decrease of 11 per cent of the original value. There was no evidence of skin edema and when the transfusion was stopped the fall in impedance ceased.

The effect of tissue edema on resistance. A very marked fall in total body impedance occurs when extensive edema is produced by infusion with isotonic salt solution (fig. 4). We are engaged in a further study of impedance in experimental and clinical edema, details of which will be reported later. At the present time there is one consideration, however, the importance of which is evident, namely, the effect of lowered body resistance on the electrocardiogram, especially when recorded by galvanometers of relatively low resistance, such as the string galvanometer. Electrocardiograms exhibiting "low voltage," a decrease in the size of the waves in all leads, have been taken as indicating in an important degree advanced myocardial degeneration. It is apparent on theoretical grounds that a decrease in body resistance will produce the same effect (21), and it is easy

to demonstrate this fact experimentally. It would seem important to question, therefore, the significance of this finding in the presence of extensive edema. A high input resistance to the recording circuit, obtained by use of an amplifying circuit ahead of the galvanometer of whatever type used, will go far toward reducing the effect of changes in resistance in the body part of the circuit.

CONCLUSIONS

The electrical field surrounding the heart in the tortoise approximates more closely that of a plane than that of a homogeneous conductor the shape of the body. A similar situation appears to exist in the mammal. Measurements of the electrical impedance along various conducting paths in dogs and rabbits show the importance of muscle as a conducting path, especially when present in compact masses and containing its normal blood complement. The blood vascular system, especially beyond the larger vessels where the extent of the conducting path is large, appears of significant importance. The approximation to a planar distribution of the principal paths of electrical conduction in the organism is believed to account for the approximation of the electrical field surrounding the heart in situ to the electrical field in a plane.

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THE RÔLE OF THE EXTREMITIES IN THE DISSIPATION OF HEAT

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It has been shown by several investigators (Benedict and Root, 1926; Soderstrom and DuBois, 1917; Johnston and Newburgh, 1930; Newburgh, Wiley and Lashmet, 1931) that at comfortable environmental temperatures and with activity short of physical labor, approximately 76 per cent of body heat is dissipated by radiation, conduction and convection from the skin, and 24 per cent by the vaporization of water from the skin and lungs. In cold weather more clothing is added as a protection against the environment. If this is not sufficient to maintain the normal body temperature the production of heat is increased. In hot weather or with the increased heat production of hard physical labor, scantiness of clothing is usually not sufficient to keep the dissipation of heat at the usual proportions. The amount lost by the vaporation of water rapidly increases and may be called upon to care for 100 per cent of the total dispersion (Wiley and Newburgh, 1931).

The temperature of the skin at any one point is a resultant of the heat brought to it largely by its blood supply and the heat dissipated from its surface by the processes just mentioned. In a previous investigation (Coller and Maddock, 1932) involving skin temperatures, it became evident that the shift of blood to or from the surface of the body to meet the required dissipation of heat under different environmental conditions was not uniform to all parts of the body. The purpose of this paper is to present evidence that the regulating shift of blood is largely to and from the extremity surface rather than simply the surface of the body in general.

The studies were carried out in a room sufficiently insulated so that temperature and humidity could be kept at any desired level. All skin temperature measurements were taken with a "Tycos Dermatherm." The points of measurement are shown in figure 1. Their distribution is largely on the extremities because their original selection was made in regard to peripheral vascular diseases. The points for the head and trunk, however, are fairly representative of those areas and the measurements obtained are in entire accord with the work of other investigators (Benedict, Miles and Johnson, 1919; Eddy and Taylor, 1931).

The study was divided into two parts.

GROUP A. *The response to increased environmental temperature.* Six normal male subjects wearing only "shorts" and lying on an examining table were exposed to an average room temperature of 25°C. The relative humidity was maintained at about 25 per cent. To permit a complete adaptation of the heat dissipating mechanism to the environment, a period of one hour was allowed before skin temperature measurements were recorded. The subjects were then covered with a light rubber sheet to the clavicular line and wrapped up in three woollen blankets. The temperature of the "private environment" under these coverings varied from 34°C. to 36°C. The humidity was not measured but was considered to be high, as the under side of the rubber sheet and the subject's skin were usually covered with moisture. The subjects remained covered for one hour before skin temperature measurements were taken. To record these as they existed under the coverings, the blankets and rubber sheet were adjusted only sufficiently to expose the desired skin point. There was no increase in mouth temperature during the covered period.

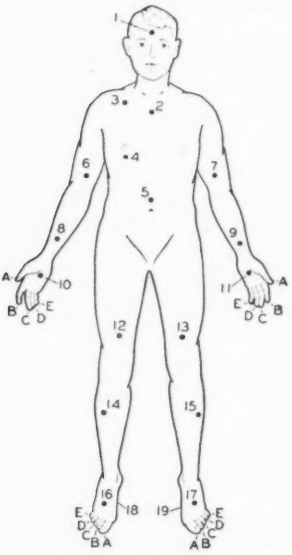

Fig. 1. Points of skin temperature measurement.

The average skin temperature measurements for the two periods are shown graphically in figure 2. The number of degrees Centigrade increase between the two conditions is shown for various body points. Of particular interest was the fact that the skin temperature of the extremities increased to a much greater extent than that of the head and trunk. The effect was more marked in the lower extremity than in the upper extremity and in both increased distally.

This result shows that the shift of blood to the surface of the body as a part of the heat regulatory response to higher environmental temperatures and humidities is not a uniform shift to all parts of the body surface but actually a much greater transfer to the extremity surface than to that of the head and trunk. Such being the case, under constant environmental conditions, increases in the production of heat ought to produce the same result.

GROUP B. *The response to increased heat production.* Two series of subjects wearing only "shorts" were used in this group. For both of them the production of heat under basal conditions was determined by indirect

calorimetry. The relative humidity of the room was maintained at about 25 per cent.

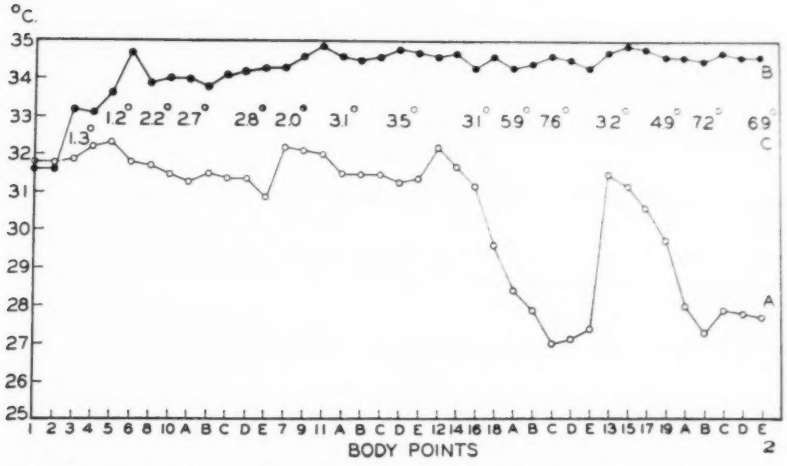

Fig. 2. Average skin temperature response of six normal men to increased environmental temperature and humidity. A: Following exposure to a room temperature of 25°C. for one hour. Relative humidity 25 per cent. B: After being covered with a light rubber sheet and three blankets for one hour. Temperature of air under coverings 34°C. to 36°C. Subjects' skin and rubber sheet covered with moisture. C: Skin temperature increase between the two conditions.

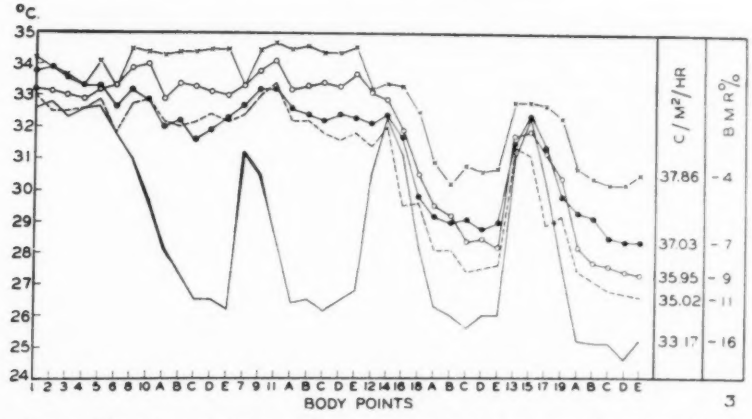

Fig. 3. The effect of the basal heat production of five normal men on their skin temperature under constant environmental conditions. Room temperature maintained at 83°F ±1°. Relative humidity 25 per cent.

For the first series, five young adult males were studied under a constant room temperature of 83°F. ±1°. Every effort was made to eliminate

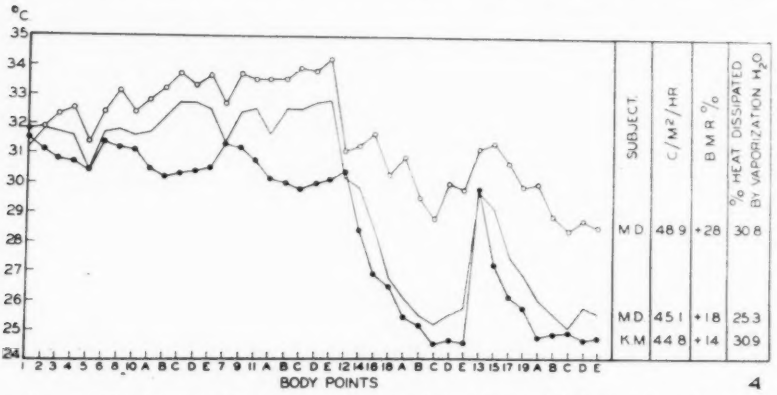

Fig. 4. The effect of the heat production of a patient with hyperthyroidism (M. D.) and of a normal subject after a high protein meal (K. M.) on the temperature of their skin under constant environmental conditions. Room temperature maintained at 76°F ±1°. Relative humidity 25 per cent. Subject M. D. studied three days before a subtotal thyroidectomy was done B.M.R. +28, and again six days after the operation, B M.R. +18.

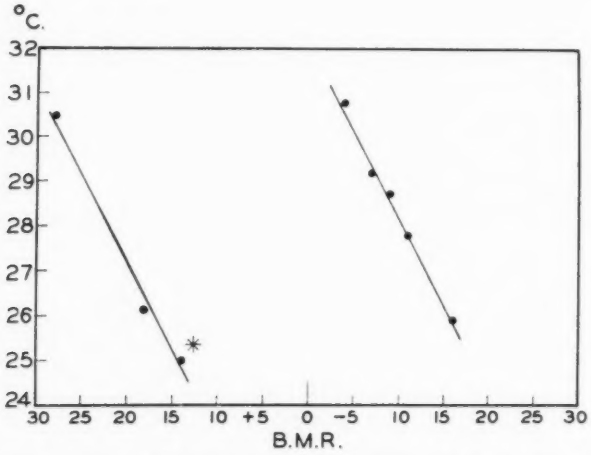

Fig. 5. The relationship of the average skin temperature of the great toes to the basal metabolic rate under constant environmental conditions. From the data of group B. Relative humidity maintained at 25 per cent. Room temperature for the minus rates kept at 83°F. ±1°, for the plus rates 76° ±1°.

* Subject K. M. not basal, metabolic rate having been increased by a high protein meal.

psychic factors. The skin temperature measurements were taken at the end of the metabolic test period.

The results obtained are shown graphically in figure 3. With the increases in heat production, increases in skin temperature occurred and were of a much greater degree for the extremities than for the head and trunk.

The metabolic rates of the subjects of the first series varied from −16 per cent to −4 per cent. For the second series, two subjects with higher rates were selected. K. M. was a young adult male whose metabolic rate was increased from a basal of −11 per cent to +14 per cent by means of a high protein meal. M. D. was a patient with an exophthalmic goiter. The metabolic rate of +28 occurred in a study a few days before the subtotal thyroidectomy was done; the +18 about a week post-operatively. The three studies of this series allowed a comparison of the data of one subject with that of the other subject and also of the same subject under different productions of heat.

It was thought advisable to know that the recorded skin temperatures which were being considered as indicative of the blood supply to the surface were those obtaining under normal dissipations of heat. Accordingly, in addition to the determination of the heat production of these two subjects, a determination of their insensible loss of weight[1] (Wiley and Newburgh, 1931) for a two hour period permitted a calculation of *1*, the amount of heat removed by the vaporization of water, and *2*, the ratio between it and the total dissipation of heat. The room temperature for these subjects was comfortable at 76°F. ±1°.

The results are shown in figure 4. The dissipation of heat by the vaporization of water varied from 25.3 per cent to 30.9 per cent, which is a normal ratio of the total dispersion of heat for studies over short periods of time (Wiley and Newburgh, 1931). These two subjects therefore were dissipating heat in a normal manner. As in the previous series, the skin temperature increases in response to the increased heat production were much greater for the extremities than for the head and trunk. Viewing the extremities alone, the increases in skin temperature were greatest in the hands, legs and feet. In general, the toes showed the most marked response and reflected the increase in heat production more than any other body point tested. The exact relationship between these two factors is shown in figure 5 where from the data of figure 3 and figure 4 the average skin temperature of the great toes was plotted against the corresponding metabolic rate. A simple linear relationship is seen to exist. This is further evidence that the shift of blood to the extremities is part of an orderly physiological process.

Under basal conditions, all energy transformation is dissipated as heat.

[1] The balance used was accurate to 0.1 gram.

The skin temperature changes shown with increased dissipations of heat under constant environmental conditions clearly indicate that the regulating shift of blood to the surface of the body occurs to a much greater extent to the surface of the extremities than it does to the surface of the head and trunk. As stated, an analogous response occurred as a result of increasing the environmental temperature.

Discussion. It is common knowledge that the hands and feet tend to swell during warm weather and shrink during cold weather. That these changes are due in part to increases and decreases in peripheral blood flow, has been shown by the plethysmographic studies of Hewlett, Van Zwaluwenberg and Marshall (1911), Stewart, (1913), Muller and Veiel (1910–11) and others. Investigations on volume changes, however, are practically limited to the extremities and do not permit the comprehensive view of shifts of blood to the total body surface as can be obtained by measurements of the temperature of the skin.

The amount of skin surface on the extremities in relation to the total area is not generally realized. Takahira (1925), using the DuBois (1915) method of measuring the surface area, showed the following distribution for one typical Japanese subject:

	Area distribution *per cent*
Head	10
Trunk	25
Arms	14
Hands	6
Legs	38
Feet	7
	100

The extremities have 65 per cent of the total. The proportion from the figures of DuBois (1915) for his subjects is slightly less. In the dissipation of heat, the regulating shift of blood to the extremities is therefore to an area sufficiently large to care for wide normal variations.

It has been shown that there is a gradient of temperature (Loewy and Dano, 1925; Bazett and McGlone, 1926a, 1926b, 1927) from the deep tissues of the extremities to the surface. When the skin is cool, the subcutaneous tissue and muscle for a depth of 3 to 5 cm. or more are at a temperature lower than that of the blood. In general, the gradients are minimized by exposure to warmth and exaggerated by exposure to cold. It is interesting that in the maintenance of the constant internal temperature of the head and trunk, there should be little change in the temperature of the comparatively thin enclosing structures, particularly when there is available extremity tissue thrust out into the environment in such a way

as to promote the dissipation of heat and also able to function satisfactorily under several degrees' variation in temperature.

There has been a controversy for years as to the mechanism by which the cooling of one hand occurs when the other hand is immersed in ice water. Pickering (1932) recently reviewed the question and presented further evidence that the cooling is not a simple reflex vascular phenomenon but is the result of a general reaction of the heat control mechanism to the cool blood returning from the hand in ice water. From the data of this investigation showing the important part played by the extremities in the dissipation of body heat, it is obvious that the application of cold or heat to an extremity must alter the general heat dissipating adjustment present just prior to the application with consequent vasoconstriction or vasodilatation of the blood supply to the remaining body surface.

The observations on skin temperature presented emphasize the importance of the extremities in the dissipation of body heat over that of the remainder of the body surface. In the same regard, from an investigation of the insensible loss of water, Loewy (1914) reports that per square meter of body surface, this heat dissipating process is greatest in the arms, next greatest in the legs and least from the trunk, the extremities yielding not far from 75 per cent of the total. Considering parts alone, the greatest actual loss occurred from the legs. There have been many studies of physiological response to heat (reviewed by Bazett in 1927) and of the heat regulatory mechanism of the body (reviewed by Barbour in 1921) which take on a greater or a new significance when the important part played by the extremities in the dissipation of heat is recognized.

CONCLUSIONS

1. Evidence regarding the volume flow of blood through the skin was obtained by means of changes in its temperature.

2. The close connection between the temperature of the extremities and the heat dissipating mechanism is shown by the fact that under constant environmental conditions a simple linear relationship was found between the temperature of the great toes and the basal heat production per unit of surface area.

3. Increasing environmental temperature or increasing heat production causes much greater shifts of blood to the integument of the extremities than to that of the head or trunk.

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STUDIES ON THE CORONARY CIRCULATION

II. The Systolic and Diastolic Flow through the Coronary Vessels

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Opinion still remains divided as to whether the force of ventricular contraction impedes or arrests the blood-flow through the intramural vessels or whether aided by the elevation of aortic pressure the flow is actually accelerated. The main views are summarized graphically by the chart of figure 1. The extensive investigations of Anrep and his associates (1927, 1928, 1929) seemed definitely to have settled the question to the effect that the flow is completely arrested during ventricular systole. But these conceptions were challenged by Hochrein and his associates (1930–31), partly on the ground that the hot wire anemometer employed by the British investigators was inadequate for accurate determinations of phasic variations in flow, partly on the basis of new experimental evidence which indicated that the maximal flow bears a very changeable relation to systole and diastole with a tendency to become maximal during systole and minimal during diastole. Under guidance of Anrep these criticisms of apparatus and results led to further evidence as to the efficiency of the hot-wire anemometer (Davis, Littler and Volhard, 1931) and also to an improvement of the apparatus (Rössler and Pascual, 1932). As a result, conclusions were reached that the criticisms offered were unjustified and that Hochrein's procedures were, to judge from the character of published records, extremely questionable. In 1931, Anrep, Davis and Volhard again summarized the main conclusions, viz., 1, that the blood-flow through the intramural vessels is either completely stopped or greatly diminished by contraction of the heart; 2, that during vigorous contractions a certain amount of regurgitation may occur, especially when the perfusion pressure is lower than aortic pressure; 3, that the flow increases progressively after the incisura and under normal conditions reaches a maximum rate some time after relaxation has been completed, and 4, that the flow remains at this maximum until the onset of the next systole.

[1] Travelling Fellow of the Rockefeller Foundation.

[2] A preliminary report of this research and a demonstration of the apparatus employed were given at the meeting of the American Physiological Society in Cincinnati, April 10–12, 1933, cf. This Journal, 1933, cv, 25.

Two subsequent discoveries are difficult to square with this interpretation. First, Anrep, Davis and Volhard (1931) themselves discovered the paradoxical fact that two forward movements of blood take place—one during ejection and another smaller forward movement during diastole—when the coronary arteries are perfused by the natural pulsatile aortic pressures. In short, this appears to agree with the observations of Rebatel

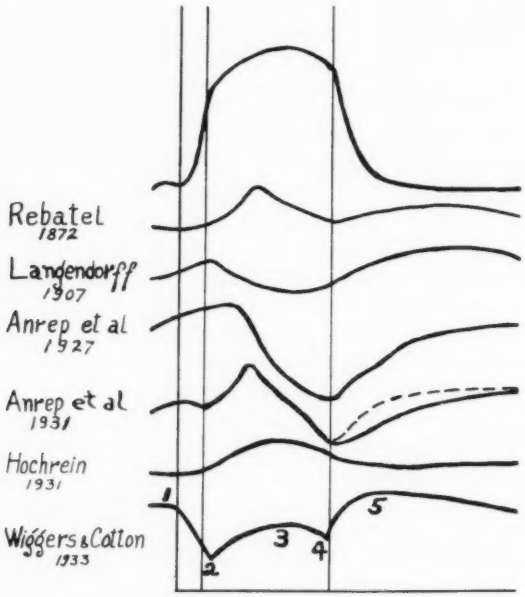

Fig. 1. Series of curves related to intraventricular pressure curve, giving approximate ideas as to views regarding variations in rate of coronary flow. Curves of Rebatel, Langendorff, Anrep et al. (1927) represent different conceptions as to rates of flow through intramural vessels. Curve of Anrep et al. (1931) represents curve of coronary inflow with pulsatile aortic pressure but not flow through intramural vessels. Curve of Wiggers and Cotton (1933) represents probable flow through intramural vessels with pulsatile aortic pressure; arrived at by indirect analysis of data in this paper.

(1872) and of Hochrein and Keller (1931), but as pointed out by the investigators themselves they seem to clash with all the results of their previous experiments. This discrepancy they explain on the basis that when blood or saline enters the coronaries under a pulsating pressure the delivery from a cannula into a large coronary branch no longer remains a criterion of the flow through the intramural vessels, for the large elastic branches by their expansion and collapse can modify to a consider-

able extent the delivery from such a cannula. This explanation deserves further experimental test, particularly in view of our recent observations (1933) that the pressure pulses recorded from the coronary artery give no evidence of such inherent elastic oscillations and furthermore showed no evidence that flow through the branches of the coronary vessels is significantly impeded during systolic ejection.

METHOD. The method used consists in perfusing a main coronary branch, such as the *ramus descendens anterior* with oxygenated Locke's solution and in some instances with heparinized blood supplied under a falling pressure head. A diagram of the apparatus is given in figure 2. The solution is delivered from a larger supplying reservoir into a smaller driving reservoir, *A*, where it is buffered by an adjustable volume of air in the

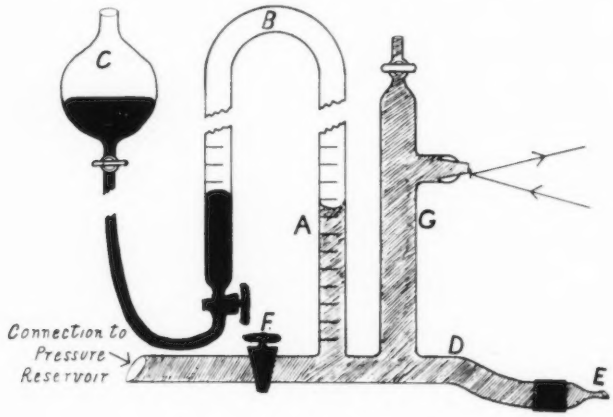

Fig. 2. Diagram of apparatus

tube, *B*. The system connects by a rigid lead tube, *D*, with the cannula, *E*, inserted into a coronary artery, attention being directed to technical details emphasized in our previous communication. Prior to each determination of flow, pressure and liquid were banked up in the driving reservoir *A* to a level considerably above systolic aortic pressure. By closing stopcock *F* quickly the fluid in the driving reservoir was driven, for a short period, into the coronary artery under a falling pressure created by expansion of air in the driving reservoir, *B*. In such a system the general gradient of the pressure decline as well as any momentary fluctuations must correspond to the rate of flow from the cannula. By keeping the capacity of the driving reservoir small (8 cc. maximum) and by introducing an optical manometer, *G*, with a high figure of merit into the system *near the cannula*, the fluctuating decline of pressure and the fluctuating flow to

which it corresponds can be recorded by means of a photokymograph. Another optical manometer is inserted into the root of the aorta and by comparison with curves so recorded precise relations of inflow rates at various moments of systole and diastole can be established. Between determinations of flow the perfusion rate is decreased to just such an extent that arterial blood reënters the perfused area from collateral branches but the orifice of the cannula is kept free from blood. In this way infusion of excessive and harmful quantities can be obviated while the area of the ventricle studied receives periodically a supply of the animal's own blood.

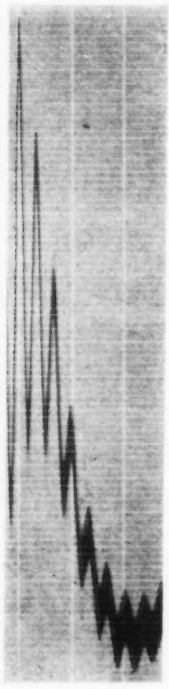

Fig. 3. Vibration frequency test

We are of the opinion that the principle embodied in this procedure is different from that employed in existing methods for determining inflow. Perfusion of an area of the heart with a pressure which falls steadily for 4 to 7 heart beats obviates the difficulty of evaluating phasic changes in flow which arise when a pulsating pressure is used. At the same time it avoids the unnatural physical conditions created when a constant perfusion pressure is used. Being the same during systole and diastole, a mean constant pressure is relatively too low during systole and relatively too high during diastole. With our method the pressure is always higher during systole. Furthermore, the use of a gradually declining pressure causes a slow elastic contraction of the larger coronary vessels, hence avoids the occurrence of brusque oscillations, such as Anrep and his associates feared might invalidate derivation of conclusions from inflow records when a pulsating stream is used. Finally the method enables one to evaluate the importance of the height of coronary perfusion pressure, within 3 to 5 consecutive beats of the heart. In this way we have better assurance that no factor other than perfusion pressure is altered.

The expedients of placing the optical manometer, which also acts as the flow recorder, near the cannula and of keeping the supplying reservoir small enhances the efficiency of the apparatus considerably. The natural frequency when the pressure is abruptly released by explosion of a rubber dam drawn tensely over the cannula is around 85 per second. In the record of figure 3 for example $T = 0.012$ second. We are not satisfied with such an attainment but believe the apparatus reasonably adequate for determination of phasic variations in flow from the cannula which are not longer than 0.06 second in duration. It is in our opinion a signal improvement over the hot-wire

anemometer which utilizes a comparatively large reservoir separated (in the early model) by a long, if rigid, tube and containing in addition a considerable volume of compressible air. Such a system cannot attain a very high natural frequency. In addition, the placement of the hot-wire device for recording flow at the extreme end of such a perfusion system is certainly a choice of necessity and not a design of choice. That a correction for lag is necessary was obviously recognized by the British investigators for such corrections are made on many published records. But the fact appears to have been overlooked that any apparatus which has such a lag also possesses too low a natural frequency to reproduce details of flow without distortion.

The records and their interpretation. Figures 4 and 5 show records obtained under a variety of experimental conditions during which heart rate, pulse amplitude and contour, and the values of systolic and diastolic aortic pressures changed considerably. The aortic and perfusion pressures obtaining at synchronous moments were determined by applying calibration scales made from tests under static conditions at the end of each set of observations. Such values are directly inscribed on records of pertinent beats, except in curve A, figure 4, in which they are easily gauged by the calibration scales at the sides. In discussing these records we shall consider first the average gradients of systolic and diastolic flow, and then the vibrations superimposed upon these fundamental gradients.

Systolic and diastolic gradients. Examination of a full record such as is shown in curve A, figure 4, shows that the perfusion pressure fell from about 140 to 20 mm., while aortic pressures measured 100 mm. systolic and 60 mm. diastolic. The extension lines drawn from beginning to end of each systole facilitate comparisons between the gradients of systolic and diastolic flows in successive beats. It is apparent that the rate of flow is always less during systole than during diastole and that the degree of systolic retardation becomes greater and greater as perfusion pressure falls, until at very low levels it becomes horizontal or even rises slightly. The former denotes complete arrest, the latter a backflow, as can be verified visually during an experiment.

Obviously, the conditions of flow which approximate those in the normal heart can only be judged by beats in which a perfusion pressure at least reasonably equal to systolic aortic pressure exists. *It is only with such beats that we are concerned in our present analysis.* Curves B and C illustrate particularly well the systolic retardation of flow in comparison with the long diastole existing in these vagal beats. Despite the fact that the perfusion pressures at the commencement of systole were rather lower than systolic aortic pressure in curve B, and much less in curve C, systolic flow is merely retarded—it is not stopped. In curve D, inscribed while the natural heart rate obtained, the systolic flow is still greater in the beat

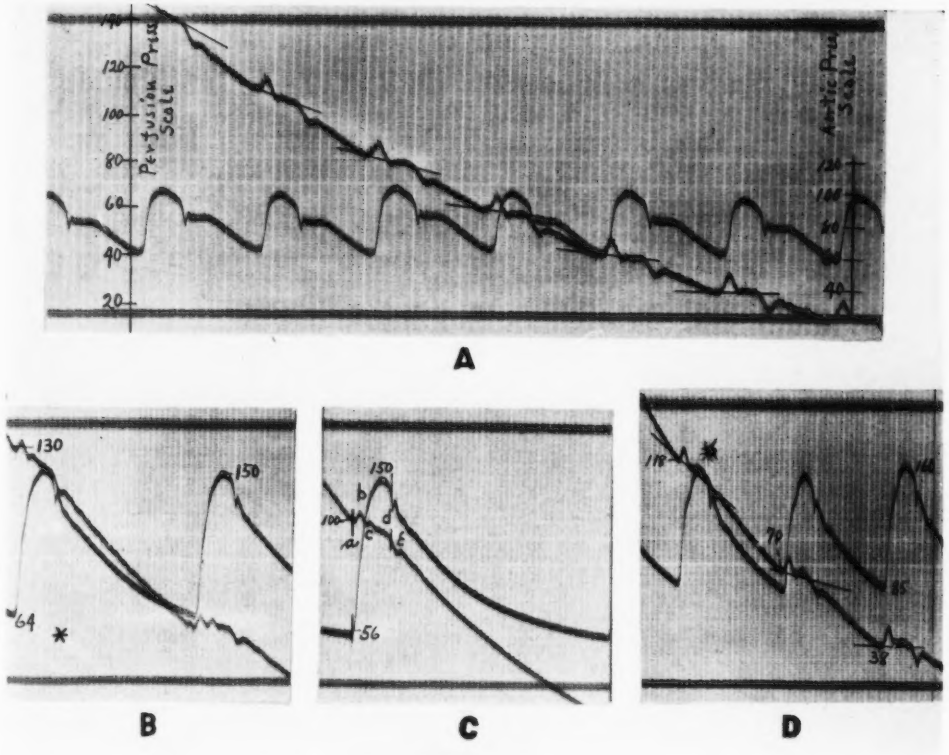

Fig. 4. Segments of records showing systolic and diastolic gradients of flow and superimposed oscillations; A, complete range of coronary pressure decline on more rapid paper; B–C, effects during peripheral vagus stimulation; D, natural rate and pressure relations following saline infusion via coronary. Discussion in text. ($\frac{1}{2}$ actual size.)

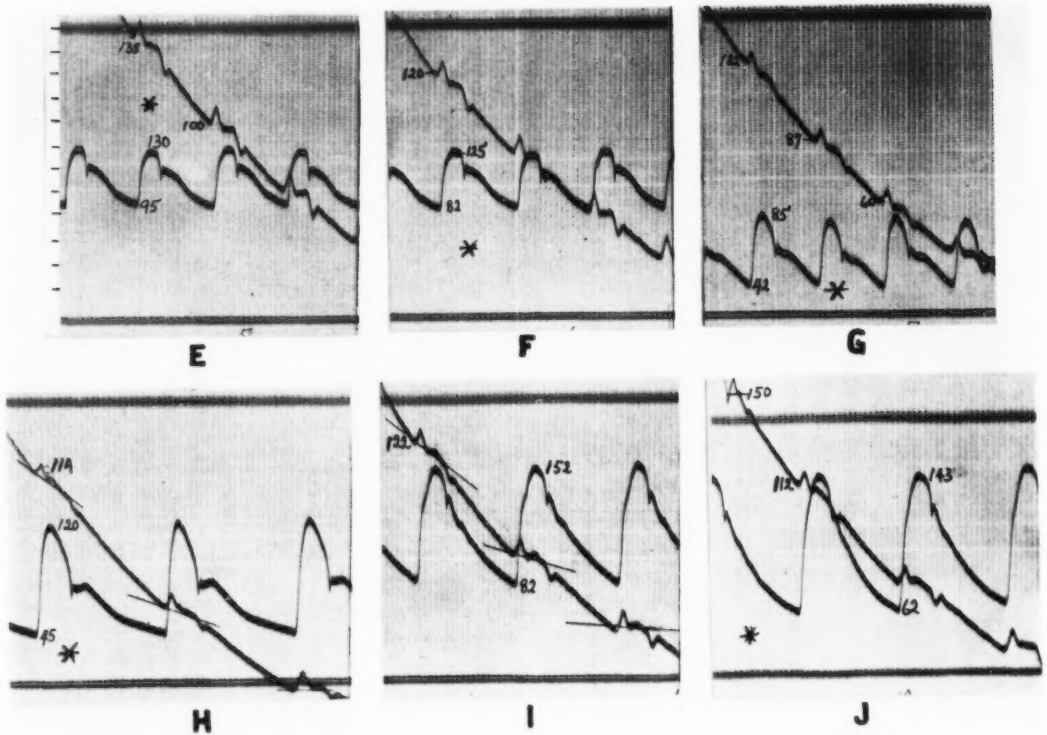

Fig. 5. Segments of records showing systolic and diastolic gradients when different relations between aortic and perfusion pressures obtain; E F, normal; G, reflex vasodilatation; H, same, with slower heart rate; I, after saline infusion; J, same, with reflex vasodilatation. Discussion in text. ($\frac{1}{2}$, actual size.)

marked (*), during which perfusion pressure more nearly equaled systolic aortic pressure. The foregoing curves were described first since they demonstrate most clearly the occurrence of a systolic flow. As a matter of fact, they were obtained after about an hour's experimentation and hence represent effects brought about by an increased systolic discharge which was the consequence of a prolonged slow infusion of saline *via* the coronary vessels.

However, a similar distinct systolic flow is also evident in curves E and F of figure 5 which represent natural conditions at the beginning of an experiment. Curve G of figure 5 shows the phasic changes in flow following reflex vasodilatation (weak excitation of the central vagus). In the first beat, the perfusion pressure is unnaturally high with respect to aortic systolic pressure and under these conditions the systolic coronary flow is reduced but little over the diastolic. The fact that flow is so little reduced despite vigorous action of the ventricles (cf. form of pressure pulses for graphic evidence) is significant. Furthermore, in the second beat of this record, systolic flow is not much retarded, although aortic systolic and perfusion pressures are nearly the same.

Curves H, I and J of figure 5 are offered as additional evidence that systolic contraction does not completely arrest the flow under experimental conditions in which the amplitude, form and actual pressures in the aorta differ widely.

The magnitude of the systolic retardation can be determined from such records by applying a volume calibration scale and determining the flows respectively during ejection and of equivalent diastolic periods immediately preceding and following such a systole. The following values in the starred beats of figures 4 and 5 are appended:

CURVE	EQUIVALENT PRECEDING DIASTOLIC FLOW	SYSTOLIC FLOW	EQUIVALENT SUCCEEDING DIASTOLIC FLOW
	cc.	*cc.*	*cc.*
B		0.33	0.50
D	61	0.33	0.49
E		0.22	0.39
F	40	0.17	0.33
G	34	0.17	0.30
H		0.33	0.50
J		0.25	0.43

Further considerations and analyses will be required before inferences can justly be drawn as to the relative decrease in flow during systole but it now appears improbable that ventricular contraction can reduce the rate of flow by more than half.

The superimposed fluctuations. Upon the fundamental systolic and diastolic gradients minor fluctuations occur. Either synchronous with or

slightly before onset of ejection, the coronary flow curve shows a transient rise followed by an abrupt fall. This initial systolic variation is apparent in all curves reproduced in figure 5, but it is marked *a*, *b* and *b*, *c* on curve C for identification. The period of this oscillation is between 0.06 and 0.08 second, i.e., at least 5 times the *natural period of the entire perfusion system.* An instrumental origin is therefore unlikely. As shown in records, the oscillation is of such form that a line drawn through its beginning and end coincides with the gradient of the curve during the remainder of systole. Occasionally a second smaller oscillation occurs on the systolic gradient. It is marked *c* on curve C and has a much shorter period than the initial vibration.

Synchronous with the incisura of the aortic pressure curve and continuing for an interval approximately equal to the period of isometric relaxation of the dog's ventricle, the coronary flow increases abruptly (*d-e*). This early diastolic onflow may approximately be styled the *incisural onflow.* The phenomenon was also recognized by Anrep and his associates, but we believe they missed the dynamic significance of the phenomenon. They refer to it as "overshooting." Inspection of our records shows that the onflow incisura dips a trifle below the diastolic gradient. While a slight overshoot therefore exists, it is but the terminal phase of the incisural onflow, is clearly produced by it and is by no means the whole phenomenon. The records show clearly that this incisural onflow serves the dynamic purpose of establishing a maximal diastolic flow *at once*, not gradually as held by the British investigators.

Discussion. It must be kept in mind that records such as we have obtained give information only as to the changing rates at which fluid is delivered by the coronary cannula into the main coronary branches. As Anrep and his co-workers have emphasized, great caution is necessary in drawing inferences regarding flow through the intramural vessels from records of inflow into a coronary artery. While we have some difficulty in accepting Anrep's theory that elastic expansion and recoil of the superficial coronary branches under pulsating pressures can be of such magnitude that the flow through the intramural vessels cannot be inferred from the cannula efflux, we agree that it is preferable to err on the conservative side rather than to draw unwarranted conclusions.

We have already emphasized our plan of avoiding conditions likely to lead to such rhythmic variations in the capacity of the large coronary branches and reiterate our belief that such capacity changes do not affect the systolic gradient of flow when perfusion pressure declines steadily, as in our experiments. Hence, our records give greater assurance that efflux gradients during systole and diastole correspond to similar gradients of flow from the peripheral coronary branches. There are, however, in addition to the intramural branches, several other sets of branches, viz.,

the collateral anastomoses with other regions of the ventricle, possibly also Thebesian communications with the left ventricle and extracardiac communications. Hence, the probability must be evaluated that the changes in flow noted may be accounted for by a run-off through these channels rather than through the intramural branches. Obviously such a collateral run-off can only occur when the pressure in the perfused branches exceeds that in branches supplied by natural pressures. It could occur therefore during systolic ejection in all beats in which the perfusion pressure during systole exceeded aortic pressures during systole; for example, it could be invoked to explain the great systolic flow from the cannula in the first two beats recorded in curve G, figure 5. It is also possible—though improbable, in view of the considerable resistance offered by collateral channels—that a collateral run-off might increase the magnitude of the recorded systolic flow in the first beats of curves A, E and J. It is inconceivable, however, that any collateral run-off occurred in the numerous instances in which a good systolic flow existed despite the fact that the perfusion pressure was somewhat below pressures obtaining in the aorta during systole (e.g., in curves B, C, D, F, H and I). On the contrary, if collateral circuits play an important rôle under such conditions of perfusion it would be more likely that a small collateral feed occurs and that the rate of flow through the intramural vessels is actually somewhat greater during systole than the inflow curves indicate. Consequently, we are of the opinion that conclusions as to changes in systolic and diastolic flow through the intramural vessels can be drawn from records of flow from the coronary cannula, provided discretion is used in the selection of beats in which proper pressure differentials exist.

With such logic as a basis, we agree with Anrep and his collaborators in a first conclusion, viz., that cardiac contraction offers an impediment to flow through the heart muscle, hence the corollary that it cannot exert a massaging action by propelling blood onward. We disagree with the conclusion that flow is completely arrested throughout systole except when the perfusion pressure sinks to unnaturally low levels. During a decline of perfusion pressure corresponding to or even slightly below aortic pressures during systole, the rate of onflow through the intramural vessels is more than half that during equal intervals of diastole.

The retardation of systolic flow probably begins *with* or very soon after isometric contraction; it is not delayed until the middle of ejection as indicated by records of the British investigators. A prompt increase of resistance is indicated by the occurrence of a supplementary initial vibration in the coronary pressure pulse (Wiggers and Cotton, 1933) and also by the preliminary oscillations (*a, b, c*) shown in flow records now presented. The rise of this oscillation (*a–b*) must be interpreted as a transient regurgitation and was also noted occasionally by Anrep and his associates. Con-

trary to their findings, it occurred consistently in normally beating hearts even when perfusion pressures exceeded aortic systolic pressures considerably; its occurrence is definitely not contingent upon the existence of a low perfusion pressure (cf. especially curve G, fig. 5).

The cause of this initial oscillation (*a*, *b*, *c*) which produces a transient backflow into the coronary cannula has not been demonstrated. The general character of the wave is consistent with the hypothesis that ventricular contraction causes a brusque increase in resistance within the intramural vessels, with the result that a sort of "water hammer" effect is produced when the previous diastolic rate of flow is *suddenly reduced*. Complete occlusion is not a requirement to produce such an effect in the elastic coronary vessels. However, other causes of such an oscillation have not been excluded. It can be assigned also to *a*, an initial emptying of the intramural vessels toward the arterial side; *b*, to mechanical compression of surface vessels during ventricular contraction, or *c*, to an unconquered artefact resulting from the sudden movement of the heart during contraction.

Whatever may ultimately prove to be the correct interpretation, it does not alter our deduction that the systolic flow is retarded almost immediately with contraction of the ventricles, for a line drawn from the beginning to the end of the oscillation (*a*–*c*) invariably indicates a slower average rate of flow than occurred during the end of a diastole immediately preceding.

As regards the process of diastolic flow we agree with the British investigators that the rate of flow is much greater than during systole. We also find that a prompt onflow into the large coronary arteries occurs with inception of diastole, but are inclined to interpret the first portion of this protodiastolic onflow from the cannula as a real acceleration of flow through the intramural vessels and not as an artefact due to overshooting. The protodiastolic dip coincides precisely with the period of isometric relaxation during which the intramural vessels are suddenly released from the compressing effect of muscular contraction. It occurs when vessels are perfused with a steadily declining pressure, i.e., under conditions where a brusque onward movement could not easily occur as a result of an increased capacity of the coronary vessels.

This brusque protodiastolic onflow serves the useful purpose of establishing a maximal flow through the intramural vessels at once. We find no evidence in any record of a gradually increasing diastolic flow which does not reach its maximum until "some time after relaxation has been completed."

The probable flow through the intramural vessels under natural pulsating pressures. In our opinion, the variations in systolic and diastolic flow through the intramural vessels when natural pulsating pressures occur in

the aorta have not so far been established by unimpeachable experimental methods. A sane application of dynamic principles to certain facts so far gathered in our experiments allows a prediction as to the variations in flow which probably do obtain under normal conditions. In a previous communication (1933) we have shown that the pressure variations in the coronary arteries conform to those existing in the aorta. We are of the opinion that three additional facts are clearly established by experiments reported in this paper, viz., 1, that the rate of diastolic flow is determined solely by the pressure in the coronary vessels, while the rate of systolic flow is affected in addition by an increased resistance in the intramural vessels due to contraction; 2, that diastolic flow reaches a maximum almost at once and decreases gradually as the pressure falls, and 3, that the resistance during systolic ejection remains fairly constant.

We are therefore justified in concluding that under natural pulsating pressures the rate of flow is also reduced during systole but not arrested completely and that a larger but gradually decreasing flow traverses the intramural vessels during the interval of diastole. We are, moreover, justified in assuming that the suddenly reduced systolic flow increases proportionally to the rise of aortic pressure up to its summit and gradually declines a little toward the end of ejection. In short, the *reduced flow* during systole probably undergoes slight fluctuations which correspond with the pressure variations in the main coronary branches, which in turn correspond to those present in the aorta. From such an indirect analysis we predict that the flow-curve in the intramural vessels under natural aortic pressures has somewhat the form shown by our curve in figure 1, rather than by that of other investigators also shown in this diagram. We would describe the changes in flow somewhat as follows:

With the onset of contraction or very shortly thereafter the flow decreases rather abruptly due to the resistance offered by compression of intramural vessels (fig. 1; *1–2*). As aortic and coronary pressures rapidly reach a systolic summit the flow increases *pari passu* (*2–3*), but the maximum reached during midsystole, or somewhat later, never equals the diastolic flow. Since aortic and coronary pressures decline somewhat during the latter portion of ejection, the rate of systolic flow decreases somewhat again (*3–4*). During isometric relaxation of the ventricles, the intramural vessels are decompressed and a sudden onflow of blood takes place (*4–5*) and the maximal rate of flow is established almost at once (*5*). During the remainder of diastole, the rate of flow decreases slowly and *pari passu* with the decline of aortic and coronary pressures during diastole.

SUMMARY

The systolic and diastolic variations in rate of coronary flow were reinvestigated by a new method which has several advantages over those

hitherto employed. Warmed oxygenated Locke's solution, and in some instances heparinized blood, was delivered under pressure from a small driving reservoir into the descending branches of the left coronary artery for a few beats at a time. The general gradient of pressure decline and its fluctuations correspond to the rate of flow from the cannula. An optical manometer was introduced near the cannula to record the fluctuating decline of pressure within the system. Tests showed that the figure of merit *of the entire system* is sufficient for these studies.

The factors which aside from changes in flow through the intramural vessels may affect the flow from the cannula are discussed and the conclusion is reached that changes in intramural flow can be inferred from changes in flow from the cannula when our procedure is used, provided discretion is used in the selection of beats in which proper pressure differentials exist.

Synchronous curves of aortic and perfusion pressures obtained under a variety of experimental conditions are presented and analyzed. When the decline of perfusion pressure during a beat corresponds fairly well with the range of pressure existing in the aorta during systole and diastole—and even when it is somewhat less—the following facts are demonstrable:

1. The systolic flow is less than for equivalent periods of diastole, either immediately preceding or following a given systole. But in no instance was a complete arrest or even an approximate stoppage of flow during systole demonstrable. This denotes that ventricular contraction increases the resistance of intramural vessels but by no means occludes them completely.

2. The rate of diastolic flow is governed solely by the pressure gradient in the large coronary branches, but the rate of systolic flow is affected in addition by increased resistance in the intramural vessels. With a progressively declining perfusion pressure the intramural resistance appears to be increased immediately and remains relatively constant for the duration of contraction, consequently the rate of flow though less than in diastole is a function of the pressure gradient.

3. With the onset of relaxation, a brusque onflow into the coronaries takes place which we call the incisural onflow. Evidence is presented that in our experiments it is not due to elastic expansion of the surface vessels but that it represents a true filling of the intramural vessels; further, that it serves the useful purpose of creating a maximal diastolic flow almost instantly.

4. Subsequent to isometric relaxation the rate of diastolic flow may be nearly twice that of the systolic flow, but it gradually diminishes as the pressure declines.

The probable changes in rate of flow through the intramural vessels when natural pulsating pressures occur in the aorta are considered. The

conclusion is reached that diastolic flow does not differ essentially from that which occurs when a gradually declining pressure is supplied. At the time of the aortic incisura a brusque filling of the intramural vessels takes place and the rate of flow becomes maximal before ventricular filling begins, then it very gradually diminishes throughout diastole in proportion to the decline of aortic pressure. The average rate of flow during systole is considerably less than during diastole, but the flow probably undergoes an acceleration as aortic pressure rises to its maximum, then slightly decreases until the end of systole. In short, the fluctuations of the reduced systolic flow probably correspond to the pressure variation in the coronary arteries and these in turn are solely dependent on aortic pressure variations.

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DISTURBANCES OF REPRODUCTION AND OVARIAN CHANGES IN THE GUINEA-PIG IN RELATION TO VITAMIN C DEFICIENCY[1]

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The effect of vitamin C on reproduction has been little studied although other vitamins have been the subject of extensive research in this connection. This investigation concerns certain effects of vitamin C deficiency upon female guinea-pigs, particularly as related to reproduction and to changes in the ovaries.

Technical papers concerning vitamin C deal most frequently with quantitative determinations or with investigation of its chemical nature. Sherman, LaMer and Campbell (1922) describe a method often followed in the assay of vitamin C, which involves the determination of the minimum protective dose. This is defined as the minimum quantity of the food, which when fed per 300 grams of body weight daily for 90 days, separate from the basal ration, will protect a standard guinea-pig from scurvy. Höjer (1926) used histological examinations of the teeth of young guinea-pigs for the quantitative determination of vitamin C. On the basis of this, Key and Elphick (1931) developed a method, the potency to be expressed in terms of a standard, as lemon or orange juice.

Vitamin C assay experiments are less rigorous than those which include growth and reproduction and few of these studies have been made with the guinea-pig as the subject. Ingier (1915) found that vitamin C deficiency in the earlier stages of pregnancy invariably resulted in premature birth or in dead fetuses, but that older animals and those in the latter half of gestation gave birth to living and apparently mature offspring. Lindsay and Medes (1926) state "that male guinea-pigs with mild chronic scurvy fail to reproduce." Goettsch (1930) reported similar results and also that "the oestrous rhythm was maintained regularly until the animal begins to lose in weight."

Reports of studies of histological changes in the tissues of guinea-pigs during scurvy are found in the literature but none have dealt with changes in ovarian tissue. Bessesen (1923) observed atrophy of the ovaries during

[1] The writers wish to thank Mr. C. G. Dobrovolny for his help in taking the photomicrographs.

scurvy. Loeb (1932) reports the parthenogenetic development of ova in the ovary of the guinea-pig.

EXPERIMENTAL PROCEDURE. Forty-three healthy young female guinea-pigs, known to be of good breeding stock, were kept in special cages in an animal room shown by investigation (Harman and Derbyshire, 1932; Harman and Prickett, 1932) to be desirable for experimental work with guinea-pigs. Each cage accommodated 7 animals, the usual number being 6 females and 1 male. The males were used for breeding only and were removed daily from the cages to eat supplementary green food. When the copulation age of the embryo was desired the male and females were kept in separate cages. They were together only when by daily inspection the vagina was found open, at which time breeding was attempted. The animals were weighed at regular intervals.

The vitamin C-free diet of Sherman, LaMer and Campbell (1922) has been modified by them and was used in the latter form:

	ORIGINAL	MODIFIED
	per cent	*per cent*
Oats, sound whole grain, ground	59	
Oats, rolled Wheat bran } equal parts by volume		59
Skim milk powder, heated	30	30
Butter fat	10	8
Cod liver oil		2
Sodium chloride	1	1

This diet has been thought to be devoid of vitamin C but adequate in other respects.

The animals were fed the basal diet and green food *ad libitum* for at least one week, to allow adjustment to new conditions. Then orange or tomato juice was substituted for the green food, usually in daily amounts of 1, 3, or 5 cc., respectively, per 300 grams of body weight. Some of the animals were fed the indicated amount per animal per day rather than per 300 grams of body weight per day.

At the termination of the experiment, when the animals died or were killed, autopsies were made and various tissues preserved for later study. Bouin's fixative was used and the ovarian tissue stained in eosin and Delafield's hematoxylin.

Since the diet did not prove as easily supplemented as anticipated it seemed advisable to investigate its adequacy in other respects. Accordingly, experiments were carried out in which this vitamin C-free diet, unsupplemented, was fed to young and to adult rats to study growth and reproduction, as it is recognized that the rat does not require vitamin C in the diet.

TABLE 1

Growth and survival of female guinea-pigs

ANIMAL		ANTI-SCORBUTIC FEEDING	DAYS ON EXPERIMENT	BODY WEIGHT		NORMAL WEIGHT† WHEN EXPERIMENT ENDED	DEVIATION FROM NORMAL AT END OF EXPERIMENT	
				Initial	Final		Grams	Per cent
				grams	*grams*	*grams*		
H-1	NP	None	14 K	356	348	410	−62	−15
H-8	P		14 K	302	310	350		
H-11	P		14 K	352	370	408		
I-3	P		26 K	287	266	392		
I-8	NP		26 K	283	196	390	−194	−50
B-2	NP		26 K	277	214	385	−171	−44
B-1	NP		29 D	239	150	345	−195	−57
G-1	NP		29 D	292	146	407	−261	−64
D-3	NP		33 D	453	281	565	−284	−50
G-4	NP		43 D	358	153	520	−367	−71
J-4	NP	1 cc. orange	25 K	460	426	560	−134	−24
J-5	P	juice	27 K	453	492	560		
J-7	P		27 K	373	444	457		
B-3	NP	*1 cc. to-	29 K	233	154	345	−191	−55
A-10	P	mato juice	34 K	234	187	380		
B-6	NP		36 K	262	201	407	−206	−51
B-5	NP		47 K	292	261	460	−199	−43
A-9	NP		51 K	252	282	450	−168	−37
H-3	NP	3 cc. orange	15 K	272	310	345	−35	−10
I-5	P	juice	15 K	327	330	385		
H-9	NP		17 K	283	300	352	−52	−15
I-4	P		22 K	298	334	390		
I-9	NP		23 K	330	380	412	−32	−8
H-4	NP		31 K	269	342	395	−53	−13
I-7	P		31 K	263	289	392		
D-6	P	*3 cc. to-	41 D	375	292	530		
E-13	P	mato juice	224 D	381	288	—		
A-6	P		229 K	357	716	—		
A-7	P		229 K	372	610	—		

Abbreviations: NP = not pregnant during course of experiment. P = pregnant during some part of experiment. K = animal killed at termination of experiment. D = animal died.

* Feeding indicated given per animal per day rather than per 300 grams of body weight per day.

† According to Bessesen and Carlson (1923)—most significant for non-pregnant females.

TABLE 1—*Concluded*

ANIMAL		ANTI-SCORBUTIC FEEDING	DAYS ON EXPERIMENT	BODY WEIGHT		NORMAL WEIGHT† WHEN EXPERIMENT ENDED	DEVIATION FROM NORMAL AT END OF EXPERIMENT	
				Initial	Final		Grams	Per cent
				grams	*grams*	*grams*		
H-2	NP	5 cc. orange	42 K	420	512	565	−53	−9
F-6	P	juice	43 K	455	581	582		
J-10	NP		43 K	476	550	600	−50	−8
H-5	P		73 K	445	487	632		
E-6	P		119 K	298	617	740		
D-9	P	*5 cc. to-	20 D	349	304	429		
A-3	P	mato juice	47 D	231	231	418		
E-14	P		70 D	347	487	575		

RESULTS AND DISCUSSION. *Growth.* The detailed observations on the growth of female guinea-pigs supplied the data shown in table 1. The animals have been grouped according to the antiscorbutic supplement provided and within the group according to the number of days on the experiment. The normal weights of the animals used for comparisons were secured from the normal growth curve of Bessesen and Carlson (1923). From the initial body weight of the animal and the number of days on the experiment the normal weight at the end of the experiment could be predicted. Data are included for animals killed after only 2 weeks on the experiment, but as Key and Elphick (1931) report "for a test of such short duration as 14 days the growth of a guinea-pig cannot be taken as any indication of the antiscorbutic value of its diet."

The negative controls, receiving no antiscorbutic supplement, became distinctly under weight. Observations made during this experiment suggest that the longer the animal is kept on this vitamin C-free diet the more subnormal the body weight becomes.

The 5 animals which received 1 cc. of the antiscorbutic supplements, an amount less than the minimum protective dose, had final body weights which were from 24 to 55 per cent below normal. Two of the animals, permitted to outlive the longest surviving negative control, had finally body weights 37 and 43 per cent below normal as compared with 71 per cent under weight for the negative control.

Four non-pregnant animals received 3 cc. of orange juice, an amount greater than the minimum protective dose. One on the experiment about 3 weeks was 8 per cent below normal weight and one on the experiment more than 4 weeks was 13 per cent under weight when killed. While the final weights of these animals were not fully normal, the differences are not great. Much the same thing can be said of the non-pregnant animals

receiving 5 cc. They made good growth during the 6 weeks they were kept on the experiment and were less than 10 per cent under weight when killed.

The animals which were pregnant at some time during the experiment were all below normal weight when the experiment terminated. The amounts under weight, affected by duration of the pregnancy, method of termination, time on experiment and amount of antiscorbutic supplement, vary and cannot be compared with each other or with weights of the non-pregnant animals.

Observations made during this experiment agree with those of other workers in that antiscorbutic supplements, namely, orange juice and tomato juice, when given in daily amounts of less than 3 cc., do not support normal growth or protect the guinea-pig from scurvy. It is often said that 3 cc. of orange juice or tomato juice per 300 grams of body weight will give complete protection from scurvy. Sherman, LaMer and Campbell (1922) reported fully normal growth in all animals that ate the basal diet well and received 3 cc. or more of tomato juice per 300 grams of body weight per day. Key and Elphick observed that 3 cc. of orange juice per day gave complete protection from scurvy. In the present investigation the weight of the animals receiving 3 cc. of orange juice per 300 grams of body weight was not fully normal according to the normal growth curve of Bessesen and Carlson. Normal growth was maintained for 6 weeks when the animals received 5 cc. of orange juice per 300 grams of body weight.

Reproduction. Table 2 summarizes the data concerning the reproduction of the animals used. For the animals which were pregnant during the course of the experiment, the time when pregnancy began, the duration of the pregnancy and the method of termination are shown in the table. A brief statement is made concerning changes in the ovarian tissue.

No pregnancy was secured on less than 3 cc. of orange or tomato juice. The pregnant animals which received less supplement than this were pregnant before the experiment started. At autopsy H-11 showed slight enlargement of the uterus. I-3, J-5 and J-7 were killed when they had been pregnant for about the same length of time. Resorption of the embryos was taking place in all cases but the amount of resorption in the negative control was definitely the greatest. Animal A-10 apparently aborted after 22 days on the experiment.

Three animals receiving 3 cc. of orange juice were pregnant when the experiment started. They were killed as indicated in the table and in each case resorption of embryos was occurring. Four females receiving 3 cc. of tomato juice became pregnant while on the experiment, but in all cases abortion occurred after 3 to 6 weeks of pregnancy. Five animals receiving 5 cc. portions of the supplements were pregnant as indicated in the table. Four of these animals aborted when the gestation period was well advanced.

TABLE 2

Data concerning pregnancy and changes in the ovarian tissue

ANIMAL		PREGNANCY OF FEMALES			OVARIAN TISSUES
		Began*	Lasted	Terminated	
			days		
H-I	NP				Ovary small with slight changes in structure
H-8	P	Before	25	Killed	Large cells around edge of ovary
H-11	P	First day	15	Killed	Beginning degeneration and atresia of follicles
I-3	P	First day	27	Killed	Greatly degenerated. Atretic follicles numerous throughout
I-8	NP				Degeneration less than in I-3. Atretic follicles numerous
B-2	NP				Compares with I-8
G-1	NP				No mature follicles. Large cells at periphery
D-3	NP				No mature follicles. Large cells at periphery
G-4	NP				No cells at periphery of ovary. No mature Graafian follicles
J-4	NP				Some disorganization
J-5	P	First day	28	Killed	Degeneration compares with I-8 in parts of ovary
J-7	P	First day	28	Killed	Degeneration compares with I-8 in parts of ovary
B-3	NP				Not preserved
A-10	P	Before	22	Abortion	Degeneration. Atretic follicles present
B-6	NP				No mature follicles. Some large cells
B-5	NP				Large cells at periphery and beginning degeneration
A-9	NP				Atretic follicles present
H-3	NP				Beginning degeneration
I-5	P	Before	?	Killed	Degeneration compares with I-8
H-9	NP				Beginning degeneration
I-4	P	Before	?	Killed	Many large cells around edge of ovary
I-9	NP				Degeneration. Degenerating cells
H-4	NP				Degeneration compares with I-8
I-7	P	First day	32	Killed	Few follicles with beginning degeneration

* "Before" indicates that pregnancy existed when the experiment started.

"First day" indicates that successful copulation took place on the first day of the experiment.

"After" indicates that the animal became pregnant after the beginning of the experiment.

TABLE 2—*Concluded*

ANIMAL		PREGNANCY OF FEMALES			OVARIAN TISSUES
		Began*	Lasted	Terminated	
			days		
D-6	P	After	22	Abortion	Beginning degeneration
E-13	P	After	25	Abortion	Not preserved
A-6	P	After	34	Abortion	Degeneration compares with I-8 in parts of ovary
A-7	P	After	41	Abortion	Degeneration compares with I-8 in parts of ovary
H-2	NP				Ovary small and beginning degeneration
F-6	P	First day	44	Abortion	Some large cells at periphery. Compares with J-5
J-10	NP				Very little degeneration
E-6	P	After	?	Killed	Large cells around edge of ovary. Atretic follicles present
D-9	P	Before	?	Abortion	Beginning degeneration. Some large cells
A-3	P	Before	?	Abortion	Some large cells at periphery. Atretic follicles present
E-14	P	Before	?	Abortion	Not preserved

In the case of F-6, abortion occurred after 44 days. Female E-6 was killed near the end of the gestation period when extreme losses in body weight indicated that abortion was about to take place. Two well developed fetuses were found. In this investigation no living full term young were produced. Two points are outstanding: First, there were fewer pregnancies than normal and no pregnancy occurred when less than 3 cc. of orange or tomato juice was fed; second, abortions or resorptions of embryos occurred in the pregnant animals.

When sections were studied, it was found that the ovaries were undergoing varying degrees of degeneration which may partly explain the fewer pregnancies, the abortions and the resorptions. In figure 1, which is a photograph of a normal Graafian follicle, the stratum granulosum and discus proligerus can be seen to be composed of cuboidal cells several layers thick. Figure 2 is a photograph of a Graafian follicle in the ovary of an animal receiving no antiscorbutic supplement. In contrast to the normal follicle it shows early connective tissue atresia and marked degeneration taking place throughout the stratum granulosum and in parts of the discus proligerus. Normal cuboidal cells may be seen immediately surrounding the ovum whereas near the point of attachment of the discus proligerus and stratum granulosum degeneration is evident. Very marked

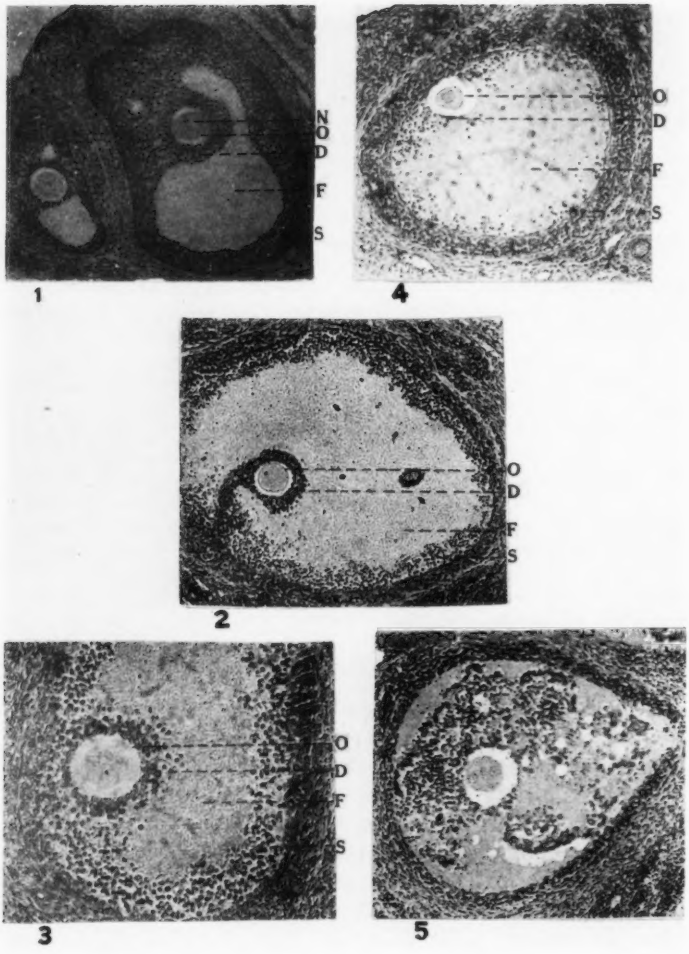

PLATE I

Fig. 1. Photograph of a normal Graafian follicle. *N*, nucleus; *O*, ovum; *D*, discus proligerus; *F*, follicular cavity; *S*, stratum granulosum.

Fig. 2. Photograph of a Graafian follicle in the ovary of an animal receiving no antiscorbutic supplement. *O*, ovum; *D*, discus proligerus; *F*, follicular cavity; *S*, stratum granulosum.

Fig. 3. Photograph of a Graafian follicle in the ovary of an animal receiving 3 cc. of orange juice per 300 grams of body weight. *O*, ovum; *D*, discus proligerus; *F*, follicular cavity; *S*, stratum granulosum.

Fig. 4. Photograph of a Graafian follicle in the ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight. *O*, ovum; *D*, discus proligerus; *F*, follicular cavity; *S*, stratum granulosum.

Fig. 5. Photograph of a Graafian follicle in the ovary of an animal receiving no antiscorbutic supplement. *O*, ovum; *F*, follicular cavity; *S*, stratum granulosum.

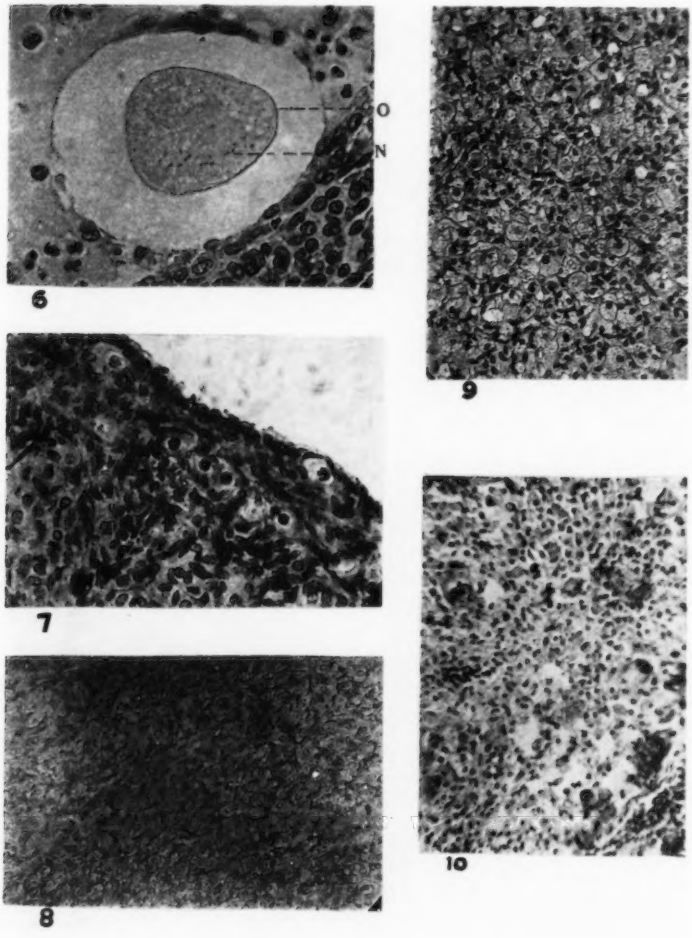

PLATE 11

Fig. 6. Photograph of the ovum in the Graafian follicle of figure 4. *O*, ovum; *N*, nuclear material.

Fig. 7. Photograph of a section through the ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight.

Fig. 8. Photograph of a normal corpus luteum.

Fig. 9. Photograph of a corpus luteum in the ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight.

Fig. 10. Photograph of a corpus luteum in the ovary of an animal receiving no antiscorbutic supplement.

connective-tissue atresia is shown in figure 5 which is a photograph of a Graafian follicle from an ovary of an animal receiving no antiscorbutic food. The stratum granulosum has completely degenerated leaving the theca folliculi next to the follicular cavity.

The changes from the normal in the ovaries of the negative controls were found to vary. The ovaries of the pregnant animals, killed after 2 weeks on the experiment, showed little change but many large cells around the periphery of the ovary. These groups of large cells (fig. 8) are arranged in long strings. Sometimes there are as many as 8 or 9 close together in a group and they appear to have no cell walls. The ovaries of the non-pregnant negative control on the experiment 14 days were small and appeared practically normal. Those animals maintained on the diet for almost 4 weeks showed degeneration extending to all of the Graafian follicles. In the ovaries of I-3, which exhibited the greatest changes, degeneration had extended to all tissues of the ovary. In the corpus luteum (fig. 10) and in the epithelial tissue degenerating cells predominated. Figure 8 is a photograph of a normal corpus luteum. Figure 9 is a photograph of a corpus luteum from an ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight. It is evident that the cells in figure 9 are fewer, contain darker staining nuclei and are more irregular than the luteal cells of the normal corpus luteum. The ovaries of the remaining negative controls contained no mature follicles. Large cells at the periphery of the ovary were found in all cases.

The ovaries of the non-pregnant animals receiving 1 cc. of tomato juice per day or 1 cc. of orange juice per 300 grams of body weight contained atretic follicles and degenerating cells in some parts of the gland. Three animals receiving 1 cc. of antiscorbutic supplement were pregnant. When the ovaries of these animals were examined, degeneration was found equal to that in the ovaries of I-8, a negative control.

Changes which were uneven throughout the ovary were evident in all of the ovaries of the animals receiving 3 cc. or 5 cc. of the antiscorbutic supplement used. When the animals were on the experiment for a short time, less change was noticeable. Figures 3 and 4 are photographs of Graafian follicles of animals receiving 3 cc. and 5 cc. of orange juice respectively. The discus proligerus in each section is more degenerated than it is in those shown in figures 2 and 3. Figure 6 is a photograph of the ovum in the Graafian follicle shown in figure 4. The nuclear material can be seen to extend throughout the cytoplasm. This is apparently due to the absence of a nuclear membrane. From a study of these ovaries no consistently greater change is found when 3 cc. of antiscorbutic supplement is fed than when 5 cc. is given. The degree of degeneration in the negative controls is greater than is found in the other ovaries.

Changes from the normal were found in all the ovaries of the animals on

this experiment. Loeb (1932) found degeneration and connective-tissue atresia in at least some of the follicles of the ovaries in which he found parthenogenetic development but many of the animals he studied were used for other experiments and at the time of death had lost a great deal of weight. Observations made during the present experiment showed structures resembling embryonic tissues which were remains of the discus proligerus and the stratum granulosum. Also, ova in different stages of maturation were found. Figure 3 shows two spindles and figure 5 shows an ovum in the two-celled stage. Lindsay and Medes (1926) found the same type of degeneration in the testis of animals on a starvation diet as found on a scurvy producing diet.

Adequacy of diet for rats. The rats fed the Sherman, LaMer and Campbell vitamin C-free diet, unsupplemented, consumed the food well, appeared to thrive, presented a healthy appearance and made good growth. The males and also the females placed on the diet at 4 weeks of age grew at least as well as the animals reported by King (Donaldson, 1924) and by the time they were 12 weeks of age all of the females had become pregnant and 3 of the 5 had borne healthy litters. The rats placed on the diet as young adults continued to grow normally and litters were born at normal intervals. To study the second generation, a few of these young were retained on the diet until they were mature. They grew at least as well as the animals reported by King and reproduced at an early age, indicating that this standard C-free diet was adequate for normal growth and for reproduction of rats.

SUMMARY AND CONCLUSIONS

1. Rats fed the Sherman, LaMer and Campbell vitamin C-free diet appeared healthy, grew normally and reared normal young.

2. Guinea-pigs receiving 1 cc. of orange juice or tomato juice could not maintain their body weights; those receiving 3 cc. grew at a slightly subnormal rate; and those receiving 5 cc. made good growth.

3. All female guinea-pigs receiving the standard diet together with supplements of 1, 3, or 5 cc. of orange juice or tomato juice failed to give birth to living young.

4. There were fewer pregnancies than normal in all the experimental guinea-pigs and no pregnancies occurred when less than 3 cc. of orange juice or tomato juice were fed.

5. Abortions or resorptions occurred in the pregnant experimental guinea-pigs. This was true whether pregnancy had taken place before the animals were placed on the experiment or whether they became pregnant during the experiment.

6. The ovaries of these animals varied from the normal in that there was a degeneration of the Graafian follicles and a lack of normal development

of new Graafian follicles. In the extreme cases of the degeneration of the Graafian follicle there was neither a discus proligerus nor a stratum granulosum.

7. The amount of variation from the normal depends upon the length of time the animal was on the diet, the amount of antiscorbutic supplement fed, and whether or not pregnancy had existed.

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THE MAMMALIAN VAGUS NERVE—A FUNCTIONAL AND HISTOLOGICAL STUDY

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Functions served by fiber groups in the mammalian vagus nerve. An analysis of the vagus nerves of the turtle and cat (Heinbecker, 1930) demonstrated three separable potential complexes. A correlation between potential form and nerve fiber structure as revealed in osmicated cross sections of nerve indicated the first potential complex to be associated with activity in the larger myelinated fibers, the second with activity in the small thinly myelinated fibers, and the third with activity in the non-myelinated fibers. The first of these fiber groups was shown to have physiological properties characteristic of somatic nerve fibers, the other two of autonomic efferent fibers. In this paper are discussed the functions which these various fiber groups of the mammalian vagus nerve serve in the bodily processes. The physiological studies have been correlated throughout with their underlying histology, the aim of which has also been to determine the locus of the cells of origin of the various fiber groups. Briefly stated, the present results establish the afferent nature of the large myelinated fibers responsible for the first potential complex other than those motor to the larynx and similar skeletal muscle structures, and confirm the efferent nature of the small thinly myelinated and non-myelinated fibers responsible respectively for the second and third potential complexes. Of these efferent fibers, some have their cells of origin within the central nervous system; others within the nodose ganglion. No synapse has been demonstrated in the nodose ganglion.

The essential feature of the physiological investigation has been the utilization of the established correlation between potential form as recorded by the cathode ray oscillograph from a nerve trunk after conduction and the nerve's fiber content. By the coincidental recording of the potential, and of the effect on the organism resultant from electrical stimulation of a nerve trunk still functionally attached, it is possible to correlate such body function with fiber group activity. The electro-neurogram, like the electrocardiogram, thus becomes an index of function. Owing to the considerable differences in threshold of the various fiber groups, it is possible

to study the functional effect of each by increasing the strength of stimulation. However, only the fiber group with the highest threshold can be investigated absolutely separately by this technique without special modifications. With increasing stimulus strength the effects of the fiber groups with lower thresholds are superimposed upon the effect from the first group. Fortunately the different fiber groups are, for the most part, associated with quite specific and easily recognizable functions. The differential study of the fiber groups is further facilitated by utilizing the results of studies by Gasser and Erlanger (1929) demonstrating that pressure blocks the large fibers in excised nerves before small ones. By applying pressure to the vagus nerve still functionally attached, it has been possible for us not only to separate out functional subdivisions within an extensive fiber group, but also to study the function of the non-myelinated fibers separately from the myelinated ones.

The functional studies included the following: a determination of the character of the fibers bearing the afferent impulses resulting in respiratory and blood pressure changes; a study of the conduction of the action potentials through the nodose ganglion centripetally and centrifugally; a potential analysis of the cervical vagus trunk after removal of the superior cervical ganglion and adequate time for functional degeneration; stimulation of the cervical vagus after section above the nodose ganglion, after allowing adequate time for degeneration (ten to twenty days), to determine the loss of effect on the heart rate, on calibre changes in the bronchi and on intestinal movements; stimulation of the cervical sympathetic trunk cut low in the neck to determine whether or not a potential can then be obtained *from the cervical vagus*, care being taken that the relationships between the superior ganglion and the vagus ganglion were undisturbed.

TECHNIQUE. Silver electrodes served for both stimulating and recording. Condenser charges were used to stimulate. The recording mechanism was the cathode ray oscillograph in association with an amplifier yielding a sensitivity up to 400 mm. per millivolt. In the studies designed to identify the afferent fibers, respiratory movements were registered through a pneumograph held by a tape around the chest and attached to a tambour. Blood pressure changes were manometrically recorded. In these experiments the vagus nerve was dissected free in the neck and cut at the point of its entrance into the thorax, leaving its central end attached. Stimulating and recording electrodes were arranged to permit the co-incidental observation of the potential record and the effects of stimulation on the animal's activity. In the efferent fiber studies changes in heart rate were determined either by counting the pulse in the exposed carotid artery or from manometric records of the arterial blood pressure. Changes in the calibre of bronchi were determined with the thorax opened. Positive pressure ventilation was applied through a specially devised tracheal can-

nula[1] arranged to permit the registration on the kymograph of changes in resistance of the bronchi to air flow during stimulation of the nerve (fig. 1). The intestinal movements were studied in the duodenum. The recording system consisted of a water filled balloon connected through a water manometer with a tambour.

Cats, rabbits and dogs were used as experimental animals. The operative procedures carried out were removal of the superior cervical ganglion, section of the vagus nerves above the nodose ganglion or section of the vago-accessory rootlets at their point of exit from the brain stem, as the experiment demanded. One side, generally the right, was left normal to serve as a control. The functional and histological studies were made ten to thirty days following the operative procedures, thus giving ample time for the development of a loss of functional activity of fibers severed from their cells of origin and also for satisfactory histological preparations.

The histological studies herein reported serve to check the physiological findings and also more specifically to investigate 1, the character of the fibers which remain in the pre- and post-ganglionic stretches when the left

[1] A recording apparatus, suggested by Dr. George H. Bishop, was designed as a pneumatic wheatstone bridge, of which two fixed arms consist of constrictions in the glass tubulature (*1* and *2*, fig. 1) and two adjustable arms are simply rubber tubes partially closed by screw clamps (*3* and *4*). Air entering in pulses from a respiration pump at the arrow escapes to the atmosphere again beyond the constrictions *3* and *4*, and the side tubes *7* and *8* passing to opposite sides of a membrane differential manometer record the balance. At arm *5* is a tracheal cannula and at *6* a rubber tube and screw cock to correspond to resistance of the air passages. The elastic lung on the one hand is balanced by a thin rubber balloon on the other. These structures are analogous to capacities with resistances in series with them in the electrical counterpart of the bridges, and are effectively in parallel to the resistances of arms *3* and *4*.

Now if, after initial balance, the value of any one element in one arm changes, as, for instance, by change in caliber of the bronchioles upon stimulation of the vagus nerve, the manometer records a shift from the base line during passage of air. It is neither practicable nor necessary to alter the corresponding opposite element (i.e., the resistance at *6* opposing the bronchiolar resistance) to obtain a new balance; the shift in the manometer reading itself indicates the direction and the approximate value of the change in resistance of the air passages when everything else is kept constant. It is not even necessary to obtain an exact balance throughout the cycle; for instance, the records show an oscillatory variation with each respiration in which it can be recognized that the change in tension on the lung did not quite counterbalance the change in tension on the balloon and the pointer thus oscillates above and below a zero line on filling and emptying of the lung and balloon. When, however, the pinch cock at *6* is screwed tighter, an upward movement of the pointer (on the record) during inflation not only shifts the base line of these oscillations, but changes their character. A constriction of the bronchioles in the opposite bridge arm should correspondingly shift the record *downward* during inflation and this is what occurs upon vagus stimulation. The record thus indicates that the result of stimulation of the vagus is to increase the resistance of the respiration passages to air. The apparatus is relatively independent of rate and depth of respiration.

nerve is severed below the jugular ganglion or intra-cranially, and 2, the distribution of fibers of the cervical and thoracic vagus to the branches to the heart, bronchi and gastro-intestinal tract, particularly those which remained following section of the trunk between the jugular and nodose ganglia, removal of the superior cervical ganglion and stellate ganglia. Much of this work was accomplished through histological study of material obtained from the animals utilized in the physiological procedures. The remainder required special investigation.

For routine histological control, each nodose ganglion with pre- and post-ganglionic stretches was drawn through a segment of spinal cord and

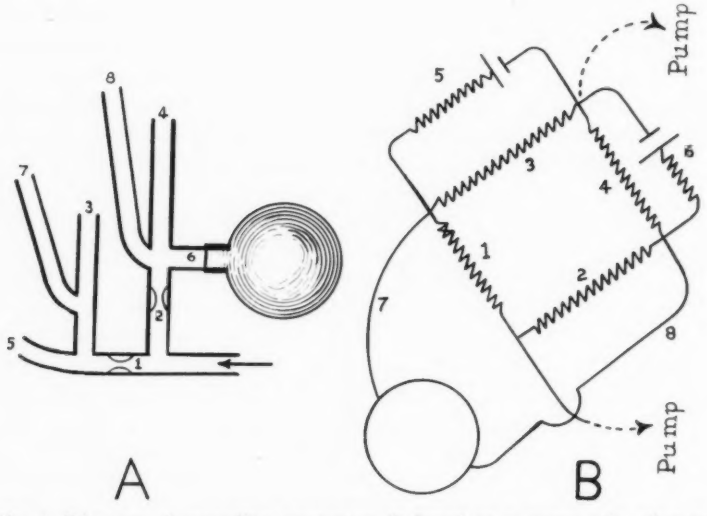

Fig. 1. Diagram of recording apparatus designed as a pneumatic wheatstone bridge to register changes in the caliber of the bronchi on stimulation of the vagus nerve. For description see footnote.

A. Apparatus as used.

B. Diagram to show analogy with a wheatstone bridge arrangement.

fixed in ammoniated alcohol (Ranson and Davenport, 1931). The cervical vagus was divided into two segments. The upper of these was affixed to a glass rod for immersion in 1 per cent osmic acid; the lower, like the nodose ganglion, was embedded in cord for fixation in ammoniated alcohol. The ammoniated alcohol material was subjected to the pyridine silver procedure. Sections showing the total fiber content of the nerve were thus provided immediately above and below the nodose ganglion and from four to six centimeters below it. The latter section was separated by an interval of not more than 4 mm. from a corresponding osmic acid cross section. In

several preparations the procedure was varied to fix the nodose ganglion with pre- and post-ganglionic stretches in osmic acid.

To obtain suitable osmic acid and pyridine silver preparations of the branches of the thoracic vagus, these were identified and dissected free of the surrounding tissue while still attached to the mother trunk. When osmic acid sections were desired, the trunk with attached branches in their proper relation was spread out on a card and fixed by immersion in 1 per cent osmic acid. After fixation and washing, the branches and also the main trunk above and below them were removed and dehydrated in separate vials. The procedure used to demonstrate the total fiber content of the branches differed slightly. As previously, the nerve and branches were removed *in toto* and spread out. The separate branches and appropriate levels of the main trunk were then cut away, drawn through short segments of spinal cord and fixed in ammoniated alcohol. These were subjected to the pyridine silver procedure.

THE AFFERENT FIBER GROUP. Experiments conducted in the manner indicated to determine the character of the fibers associated with respiratory and vaso-motor reflex effects definitely established that maximum effects for respiration and blood pressure changes were attained on activation of the fibers giving rise to the later portion of the first potential. The earliest portion of this potential is derived from the motor fibers to the larynx, etc. These fibers leave the vagus trunk in the recurrent laryngeal nerve. No further effects on either respiration or blood pressure are obtained on also stimulating the fibers giving rise to the second and third potential complexes. Interpreting the results in terms of fiber groups they indicate that the group of larger myelinated fibers convey the afferent impulses responsible for these reflexes. The threshold, conduction rate and absolutely refractory period value for these fibers (Heinbecker, 1932) definitely place them in the somatic fiber class. The fibers are shown in figure 5 H, where intracranial section of the vagus nerve carried out thirteen days previously has eliminated the motor fibers to the larynx. The depressor nerve fibers and the fibers in the vagus branches to the lung (rabbit) are shown separately in figure 6 (C and D).

The effects on respiration obtained on stimulation of vagus afferent fibers in cats have been considered previously (Heinbecker, 1932). At that time it was stated that the results could be interpreted as indicating that the activity of these fibers appeared to stimulate a center inhibitory to the natural rhythmic activity of the respiratory centers. The electrical activity of these fibers during normal respiration and in asphyxia with the lungs distended has also been recorded (Heinbecker, 1932, loc. cit.). These afferent impulses arise during the periods both of expansion and contraction of the lung; that is, during the whole period when tissue tension changes occur within the lung. During a period of continuous stretching (trachea

clamped in inspiration) a continuous flow of impulses ascends the vagus nerve.

Activity in the afferent fibers of the vagus responsible for reflex changes in blood pressure usually causes a rise of blood pressure followed by a fall. For reasons discussed in more detail in a paper by Bishop, Heinbecker and O'Leary (in press) this is interpreted as indicating the presence of both pressor and depressor fibers, rather than a double action of the same fibers. On stimulation of the saphenous nerve of the cat a pressor effect is obtained from stimulation of the larger fibers of the first group whereas a depressor effect is obtained from fibers of the pain fiber size. This is consistent with the histological finding that the myelinated fibers of the depressor nerve are mostly of the pain fiber size range. Detailed threshold studies have not been carried out on the vagus and depressor nerves, but it is evident from studies on other nerves that pressor and depressor fibers are of the same physiological type. Spontaneous activity of these fibers has been recorded both from the vagus and depressor nerves. Impulses ascend during both systole and diastole, most marked at the height of systole. Our results thus agree with those reported by Bronk and Stella (1932).

Elsewhere, Heinbecker, Bishop and O'Leary (1932) have shown that pain is the result of impulses conveyed by myelinated somatic fibers 6 to 3 micra in diameter. Experiments were carried out in cats, artificially ventilated, in which the vagus nerve was stimulated below the point of origin of the recurrent laryngeal nerve and after recovery from the anesthetic required to make the preparation. The animals react to this stimulation as animals do to painful stimuli. The stimulation required is of longer duration than is necessary to arouse pain by stimulation of either the splanchnic or saphenous nerves. This is interpreted as indicating a paucity of pain fibers in the vagus nerve as compared to the other nerves. Central accommodation to the affective quality of the sensory vagus stimuli is exceedingly marked and rapid in its development.

To further test the conclusion that all afferent fibers of the vagus are of the myelinated somatic type, pressure was applied by an especially constructed parallel jawed clamp to the vagus nerve with its central connection intact. Stimulating and recording electrodes were so arranged that the action potential could be observed on the face of the oscillograph coincidentally with the bodily reactions. In both the cat and the rabbit it was possible to block the first and second potential complexes and leave a well developed (80 per cent or better) third potential. Activity in the fibers responsible for this potential, the non-myelinated fibers, produced no evidence of pain and produced no respiratory or blood pressure reflex effects (fig. 2).

A satisfactory confirmation of the reliability of pressure as a differential agent was obtained during these experiments. It was found that in both

the cat and the rabbit the respiratory effects were depressed earlier than the depressor blood pressure effects. In the rabbit, as shown in figure 2, it was possible to eliminate all reflex respiratory effects and still have a definite effect on blood pressure remaining. If pressure blocks large fibers before small ones it should follow that the afferent fibers from the lung initiating respiratory reflex changes should be of larger size than those effecting depressor blood pressure changes. The depressor nerve contains vaso-motor afferent fibers *par excellence*. When comparisons of osmicated cross sections of lung branches and of the depressor nerve of rabbits are made (fig. 6, C and D) it is readily seen that in the former the myelinated fibers are larger than in the latter.

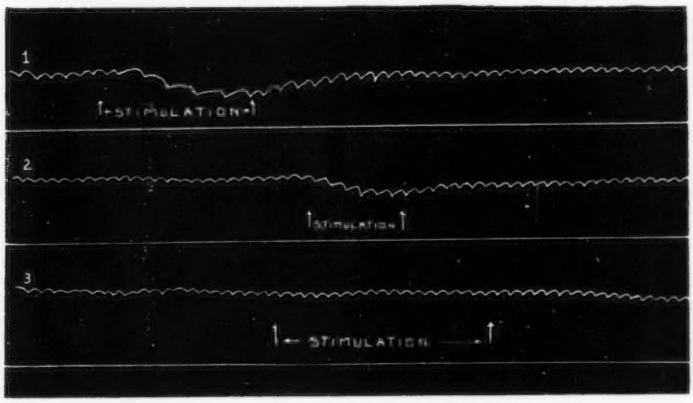

Fig. 2. *1.* Blood pressure tracing from rabbit showing depressor effect and respiratory changes on stimulation of central end of cut vagus nerve trunk in neck.

2. Same after applying pressure to vagus trunk between stimulus and brain. Note residual well marked blood pressure effect with slight residual effect on respiration.

3. Same after pressure adequate to block all of the first fiber group giving rise to the first potential complex. A well marked C potential was still present. Note there is now no effect on either blood pressure or respiration.

To summarize briefly, of the afferent myelinated fibers of a cat vagus nerve, some, 10 to 4 micra or less are concerned in respiratory reflexes, some, 8 to 3 micra in vaso-motor reflexes; the depressor fibers ranging from 6 to 3 micra which is also the range for the few pain fibers in the nerve. The afferent fibers in the recurrent laryngeal nerve range in size from 8 to 2 micra. No non-myelinated afferents are found in the vagus nerve.

THE EFFERENT FIBER GROUPS: *Conduction of the action potential through the nodose ganglion in two directions.* In a previous study of the superior cervical sympathetic ganglion of the turtle (Heinbecker, 1930) it was demonstrated that when fibers synapse in a ganglion the fact can be recognized

by a synaptic delay, definitely measurable for each fiber group with the oscillographic technique. It was also shown that on retrograde conduction through the ganglion the potential of those fibers which show a synaptic delay in the normal direction is blocked. Similar conduction studies through the mammalian nodose ganglion establish an absence of synaptic delay of any of the potential complexes pre- to post-ganglionically. In a typical experiment measurements of the conduction rate of the C potential through the ganglion at conduction distances of 50, 40 and 28 mm. gave a constant value of 0.9 meter per second. The conduction rate for this potential in a stretch of the nerve beyond the ganglion was also 0.9 meter per second. If there were a synapse present in the ganglion the apparent conduction rate should have increased for the shorter distances because the synaptic delay would form a relatively larger proportion of the total measured conduction time. On retrograde conduction there is no loss of any of the potential complexes as would be expected if one of them resulted from fibers synapsing in the ganglion. The areas of the potentials recorded from the pre- and post-ganglionic stretches are not always equal (fig. 3). This is to be expected as the nerve trunk is not the same size pre- and post-ganglionically so that the relative proportion of the total area occupied by the various fiber groups doubtless varies in the two regions. Under the circumstances a comparison of the potential areas of the various complexes is not critical for conclusions as to the number of fibers in the various groups pre- and post-ganglionic.

Sympathetic fibers in cervical vagus nerve trunk. The possible contribution of sympathetic fibers to the vagus was studied functionally, because of the assertion by Kiss (1931) and others that a large number of non-myelinated fibers contributing to the cervical vagus originate in the superior cervical sympathetic ganglion. If such were the case it should be possible on stimulation of the partially freed cervical sympathetic chain to record an action potential from the cervical vagus trunk. Experiments in which the sympathetic trunk was adequately stimulated low in the neck, with the recording electrodes on the cervical vagus, gave no evidence of any activity in the vagus fibers. Special care was taken to leave undisturbed the upper part of the superior cervical sympathetic trunk, the superior cervical ganglion, the ganglia of the vagus and the upper portion of the vagus trunk in order that there could not be any interruption of normal pathways between the two systems.

In other animals the upper part of the cervical sympathetic chain and the superior cervical ganglion were removed on one side. After adequate time for functional degeneration the action potentials of the vagus nerves on the two sides were compared. No evidence of a loss in area of any of the three potentials was obtained. This indicates that no appreciable loss of fiber content in the vagus nerve of the operated side had occurred after removal of its source of sympathetic fibers.

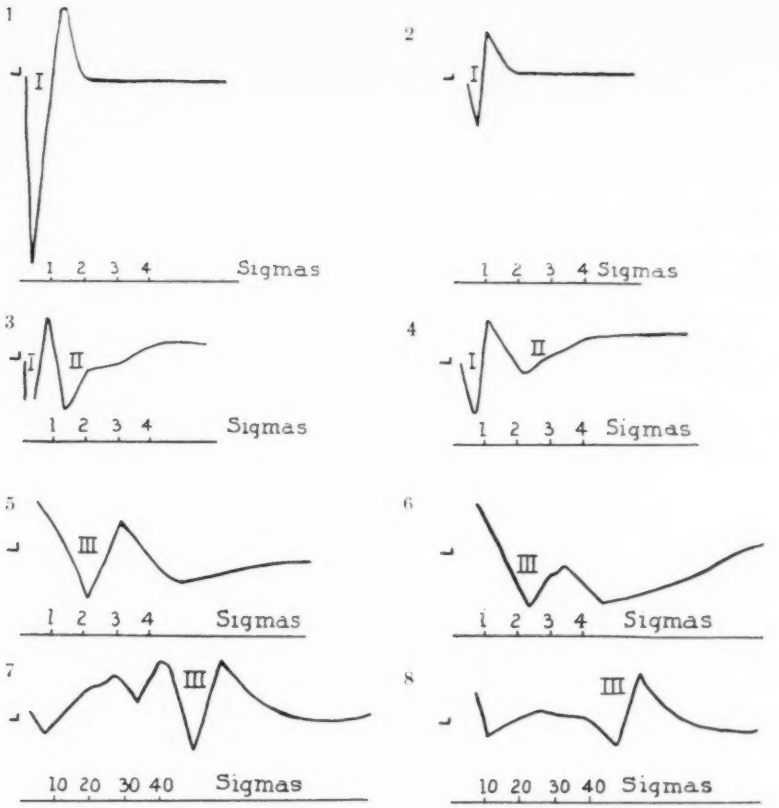

Fig. 3. *1.* Potential record normal right vagus nerve showing first potential complex. Conduction distance on the nerve 26 mm. Time in sigmas in this and subsequent records.

2. Potential record left vagus nerve cut above nodose 16 days previously. Conduction distance and amplification same as in *1.* Note the loss of first portion of potential complex known to be derived from somatic motor fibers to larynx.

3. Potential record normal control vagus nerve (same preparation as for *1*) showing both I and II potential complexes. Conduction distance on nerve 26 mm.

4. Potential record left vagus nerve (same preparation as for *2*) showing both I and II potential complexes. Note the diminution in amplitude of second potential complex. Conduction distance on nerve and amplification same as for *3.*

5. Potential record right control vagus nerve (same preparation as for *1*) showing third or C potential complex. Conduction distance on nerve 26 mm.

6. Potential record of left vagus nerve (same preparation as for *2*) showing third potential complex. Note about 10 per cent reduction in area due to loss of nonmyelinated fibers. Conduction distance on nerve and amplification same as for *5.*

7. Potential record normal left vagus nerve to show conduction of third potential complex through the nodose ganglion post- to pre-ganglionically. The deflection just ahead of the main deflection marked III may be disregarded because it results from the ganglion itself serving as a ground lead ahead of the ground lead distal to the ganglion. Conduction distance on the nerve, 40 mm.

8. Potential record from same preparation as for 7. Conduction is now through the ganglion pre- to post-ganglionically. Conduction distance on the nerve and amplification same as for 7. The area of complex III is less in *8* than in 7, the significance of which is explained in text.

It may be said, therefore, that we have been unable to obtain any functional evidence to indicate that the fibers of the cervical vagus trunk are sympathetic in origin. Similar evidence has been presented by Ranson and Mihalik (1932). Evidence has been presented by others to indicate that in the region of the stellate ganglion fibers may for a short distance accompany the vagus trunk. This is not a point of issue in the present study.

Functional studies after section above the nodose ganglion. Functional studies of efferent fiber effects were carried out on the vagus nerve after section above the nodose ganglion and after sufficient time for degeneration.

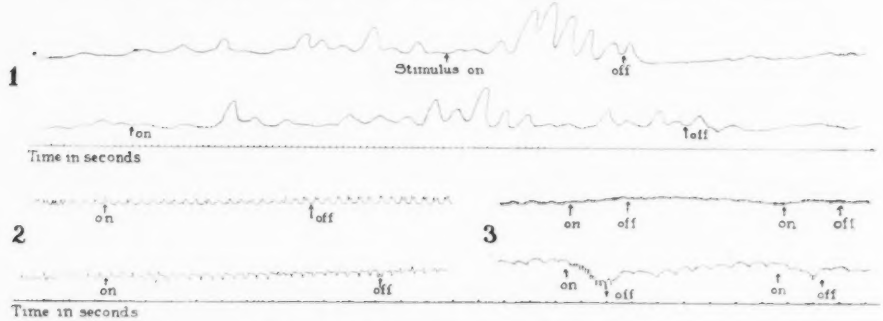

Fig. 4. *1.* Tracings of kymographic records of cat's duodenal contractions and their augmentation as result of adequate stimulation of the left vagus nerve on the neck. Upper tracing from normal animal; lower tracing from animal in which left vagus nerve had been cut above nodose ganglion 20 days previously.

2. Tracings of kymographic records showing effect of vagus nerve stimulation on caliber of bronchi. Upper tracing observed on stimulation of normal right vagus, lower tracing observed on stimulation of left vagus nerve cut above the nodose ganglion 14 days previously.

3. Tracings of kymographic records showing effect of vagus nerve stimulation on heart rate. Upper tracing effect on stimulation of left vagus nerve in neck 14 days after section above nodose ganglion, lower tracing effect of stimulation of normal right control nerve. Note the slight rise in blood pressure in upper tracing resulting from stimulation of the cut vagus.

Stimulation of the cervical vagus trunk then failed to result in any modification of the heart rate. It was still possible to secure constriction of the bronchi and either excitation or inhibition of peristalsis in the duodenum (fig. 4). In normal animals it was demonstrated by a correlation between potential form and physiological effects in the organism that constriction of the bronchi and intestinal motor excitatory or inhibitory effects are produced by impulses conveyed by the non-myelinated fibers of the vagus. One must conclude, therefore, that there are fibers in the vagus nerve whose cells of origin are in the nodose ganglia which are efferent in type. Other

vagus fibers, certain of those to the heart, are of similar character, but their cells of origin are within the central nervous system.

To determine the relative number of these fibers the conducted action potential of the vagus nerve trunk recorded from above the origin of the recurrent laryngeal nerve, twenty days after section above the nodose ganglion, was compared with the action potential of the normal vagus nerve of the opposite side. Certain differences were quite apparent. There was an absence of the potential derived from the largest myelinated fibers, known to comprise the motor element of the recurrent laryngeal nerve. There was no appreciable diminution in amplitude of the later portion of the first potential complex derived from the other myelinated fibers with somatic properties in this nerve and regarded by us as afferent. There was a diminution of 30 to 50 per cent in the second potential complex, derived from small thinly myelinated fibers with axons of the autonomic type. There was a slight diminution in area of the third potential complex, probably not more than 5 to 10 per cent. This is probably within the limit of variation between nerves on opposite sides. Consequently, it can be said that the majority of fibers of each group in the vagus nerve trunk below the nodose ganglion arise from cells in the nodose ganglion.

In a previous report (Heinbecker, 1931) it was shown that in the turtle the fiber groups responsible for the initial inotropic and chronotropic effects on the heart are different. The fibers responsible for initial inotropic effect are of lower threshold and faster conduction rate than those for the initial chronotropic effects. Those responsible for chronotropic effects are undoubtedly non-myelinated. It is considered probable, for reasons previously presented, that the initial inotropic effects are produced by small thinly myelinated fibers. In one cat the fibers conveying the impulses responsible for motor effects on the heart were found in a separate trunk from the main vagus trunk. A cross section of this nerve showed all three fiber types typically found in autonomic nerves. It is considered as evidence not inconsistent with the interpretations given in a previous paper to the effect that both myelinated and non-myelinated fibers are efferent to the heart. After section above the nodose ganglion it was shown that both small myelinated and non-myelinated fibers are lost from a restricted area of the cervical vagus, a finding which again harmonizes with our other evidence that the vagus inotropic effect on the heart may be mediated by myelinated and the chronotropic effect by non-myelinated fibers. So far, we have failed to associate any other function with the small myelinated autonomic fibers in the vagus, but we have not studied secretion of glands. Because of the similarity of their physiological properties to those of other proved efferent autonomic fibers and from the fact that all known afferent functions can be associated with fibers of another type, it is a foregone conclusion that subsequent investigations will associate them with efferent function.

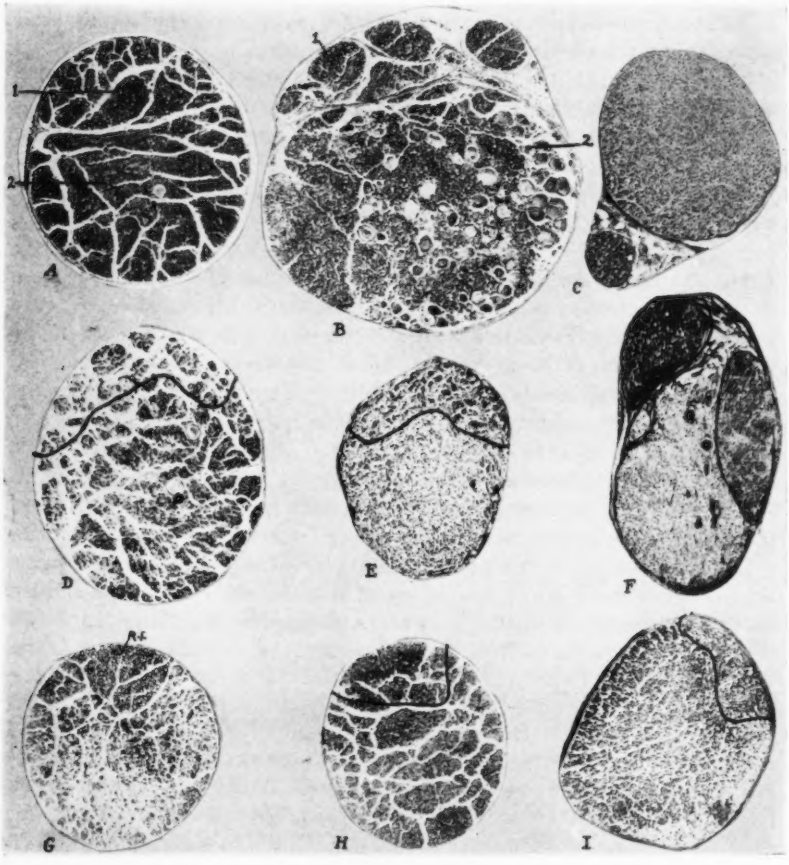

Fig. 5. Photomicrographs of cross-sections of normal and experimental left vagus nerves (cat) at various levels (osmic acid and pyridine silver preparations).

A. Normal, osmic acid, midway between the jugular and the nodose ganglia. A division of fibers into two distinctive anatomical groups is indicated (*1* and *2*). *1* essentially contains large and small myelinated and non-myelinated fibers. *2* contains small and medium sized fairly closely packed myelinated fibers, also non-myelinated.

B. Same nerve at the upper pole of the nodose ganglion, osmic acid preparation. Fascicles corresponding to *1* have now separated from the main trunk and pursue a separate course outside of the ganglion.

C. Pyridine silver preparation of normal nerve at a level corresponding to that of B. The areas corresponding to *1* and *2* in A and B indicated.

D. Upper pole of the nodose ganglion, osmic acid preparation twenty days following section of the nerve in the interganglionic stretch. Area of obvious degeneration outlined. It corresponds to *1*, A, B, C.

Effect of section of vagus nerve at point of origin from medulla. Stimulation of the vagus nerve after section of its rootlets at their origin from the medulla and adequate time for degeneration again fails to result in any effect on the heart. This indicates that fibers responsible for inotropic and chronotropic effects on the heart arise from cells within the medulla and not from those of the jugular ganglion.

A comparison of the action potential record from the cervical vagus trunk on the operated and non-operated sides reveals a loss of potential area in the second and third complexes comparable in degree to that obtained after section above the nodose ganglion. The first potential complex shows a loss of its first portion known to be derived from the largest myelinated fibers. The second portion of the first complex is unchanged indicating no loss of afferent fibers. These results correspond to the observation by Chase and Ranson (1914) that the fibers with cells of origin in the jugular ganglion pass into the upper branches of the vagus, above the level at which our potentials were recorded. They do not decide whether all such fibers are afferent.

HISTOLOGICAL STUDIES. For convenience in recording the effect upon the fiber content of the vagus nerve of the various operative procedures, those results which relate to the interganglionic stretch between the jugular and nodose ganglia, to the cervical vagus, and to the thoracic vagus and its branches will be discussed separately.

1. *The interganglionic stretch.* At a level just above the nodose ganglion a separation of the fibers of the vagus trunk into two distinctive anatomical groupings is in evidence (fig. 5, B, *1*). *One* is composed of several fascicles which contain a sprinkling of the largest myelinated fibers in the nerve, a few medium-sized ones, fairly many small myelinated, and clear areas which correspond in pyridine silver preparations at a similar level to the position of groups of non-myelinated fibers. These fascicles typically pursue a course outside of, but adjacent to, the ganglion and join the nerve below it in a region occupied in part by fibers destined for the recurrent

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E. Pyridine silver preparation following section of the nerve above the nodose ganglion. Area of obvious degeneration outlined. It corresponds also with *1*, A, B, C.

F. Areas of degeneration (outlined) immediately above the nodose ganglion when the nerve is cut intracranially.

G. Normal cervical vagus 4 cm. below the nodose ganglion, osmic acid preparation. Area labelled *R.l.* contains the recurrent laryngeal fibers.

H. Same level as G, osmic acid preparation, thirteen days following intracranial section of the nerve. Note that the outlined area of obvious degeneration includes the area labelled *R.l.* in G.

I. Pyridine silver preparation at level corresponding to G and H, twenty days after section of the nerve above the nodose ganglion. Magnification of each section 90 diameters. Zeiss 16 mm. Homal I.

laryngeal nerve. The similarity of this anatomical group to Molhant's "bulbar accessory" group is, therefore, apparent (Molhant, 1912).

The second group (fig. 5, B, *2*) enters the nodose ganglion and consists of closely packed fibers of varying size. In this group medium-sized and small myelinated fibers predominate. The largest myelinated fibers are few in number. Pyridine silver preparations of this division show fibers which on a size basis are undoubtedly non-myelinated. These occur interspersed between the myelinated fibers or in groups. Above this level the separation of the two groups is also evident in some specimens (fig. 5 A), but the line of separation is not so sharp and considerable variability is experienced. Figure 6 A and B represents higher magnification of the areas of figure 5 A, *1* and *2*. Single ganglion cells or small groups of them are observed in areas of the trunk corresponding to each fiber area. The nerve may approach the nodose ganglion as a single trunk or as several bundles.

Specimens of the vagus nerve were prepared by the osmic acid and pyridine silver methods, after interruption at a sufficient distance craniad to the nodose ganglion to allow the sections immediately above the ganglion to be relatively uninfluenced by retrograde degeneration. After adequate time for degeneration these preparations showed that the maximum degeneration occurs in a restricted area (or areas) of the nerve corresponding to the position occupied by the fiber group designated *1* in figure 5 B (see fig. 5, D and E). Some non-myelinated fibers persist and occasionally normal appearing small, medium and large sized myelinated fibers also. Even the non-myelinated fibers are not always apparent. Elsewhere, below the origin of the pharyngeal branch, areas of degeneration are not constantly observed. This does not mean, however, that single fibers were not indiscriminately removed elsewhere. Such removal is difficult to detect.

Two specimens in which the vagus nerve had been cut intracranially seven and nine days previously were prepared by the pyridine silver procedure. At a level above the nodose ganglion, corresponding to that described above, the same group of fibers as shown in figure 5, B, *1* are found degenerated. The area of degeneration appears to be of approximately the same size as that observed when the nerve is cut in the inter-ganglionic stretch. In the nine day specimen a large majority of fibers in this restricted area, including the non-myelinated, have fragmented or disappeared (fig. 5, F). Groups of intact fibers remain, however.

Fig. 6. A. Photomicrograph at 300 diameters of *1*, figure 1, A.

B. Photomicrograph at 300 diameters of *2*, figure 1, A. Note the difference in average size of the largest myelinated fibers in each of the above. Zeiss 16 mm. Homal I.

C. Photomicrograph depressor nerve of rabbit, osmic acid stain, 400 diameters. Compare the average size of sensory myelinated fibers with figure 6, D.

D. Photomicrograph bronchial ramus of rabbit, osmic acid stain, 400 diameters.

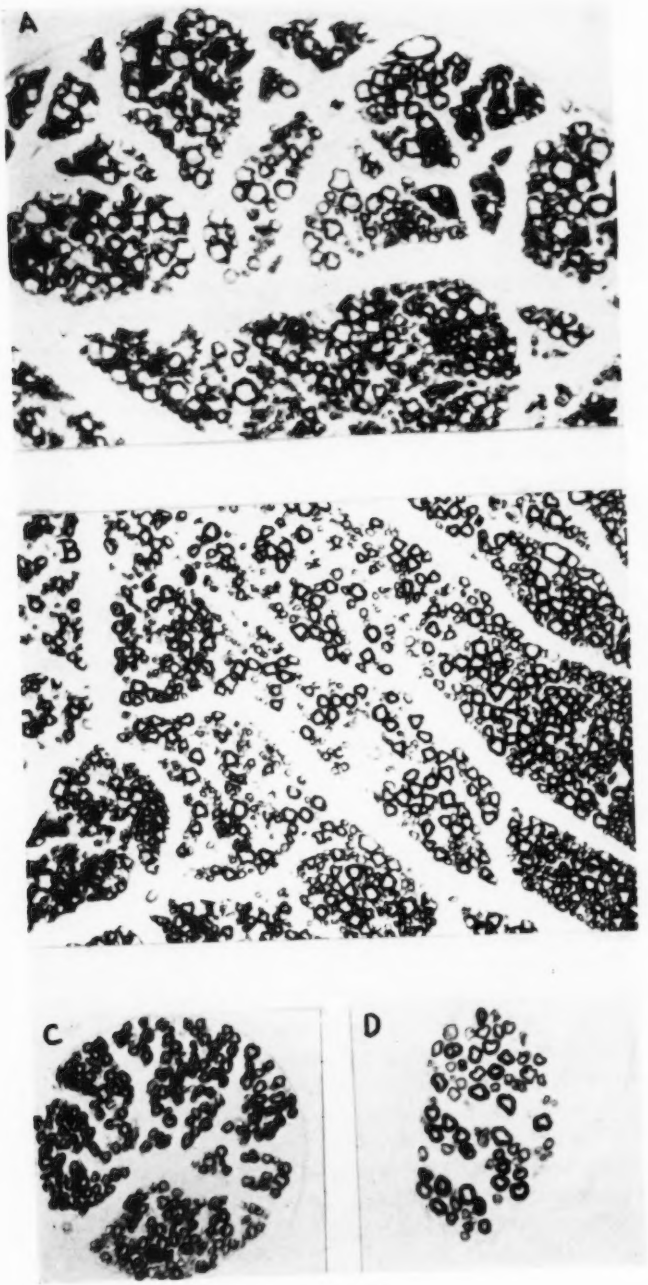

Fig. 6

2. *The cervical vagus.* The fiber distribution in the cervical vagus trunk of the cat as revealed in osmic acid prepared material is well known (fig. 5, G). Briefly, in our material the major area of the nerve is occupied by scattered myelinated fibers ranging in size from 2 to 11 microns. Well-fixed nerves reveal between these, bundles of closely packed non-myelinated fibers (pyridine silver). Toward one surface there is usually found a localized group of large myelinated fibers, many of which attain a size of 11 to 13 microns. Interspersed between the latter may occur other myelinated fibers of smaller size. When non-myelinated fibers occur in this portion of the nerve, they are found in islands intermingled among the larger myelinated ones. The area of large fibers is sometimes, in fact, separated into two or more lesser aggregates by portions of the nerve containing non-myelinated and smaller myelinated fibers. The fiber distribution in this area depends both upon individual variability in animals and variability in the level at which the section is taken. As will be indicated below, this area in the cervical trunk probably corresponds fairly closely to the bundle of fibers that passes separately around the nodose ganglion.

When the vagus is completely interrupted above the nodose ganglion or intracranially, sparse degeneration is observed except in the area occupied in part by the fibers of the recurrent laryngeal nerve (fig. 5, H and I). Here degeneration is extensive. The largest myelinated fibers completely disappear and all that remain are a few scattered myelinated fibers of medium to small size and occasionally non-myelinated fibers (pyridine silver preparations). The sharpness with which this area of degeneration is delimited from the remainder of the nerve varies from one preparation to another. In some cases it is quite circumscribed affecting almost all fibers; in others, the area of degeneration grades into apparently normal nerve, and non-myelinated and small and medium-sized myelinated fibers are frequent in the outskirts. Again the variability depends somewhat upon the level of the nerve from which the sections are taken. The ratio of the area of degeneration to the remainder of the nerve varies from 1:4 to 1:6 (pyridine silver material).

Elsewhere in the nerve osmic acid preparations infrequently reveal visible evidence of a loss of fibers. In pyridine silver material, however, small loci of degeneration are observed. The frequency of these varies from preparation to preparation; sometimes they cannot be demonstrated at all. The extent to which the loci are apparent does not depend upon the distance above the ganglion that the nerve is cut; however, the chance that trauma to the ganglion may have produced the more severe effects observed cannot be ruled out. Besides the definite loci of degeneration it is possible that single fibers are indiscriminately removed without leaving visible evidence of their elimination.

The source of that portion of the non-myelinated fibers which lie in the

same area as the recurrent laryngeal fibers is not completely evident from our material. It has been stated that in some normal nerves, non-myelinated fibers in significant numbers may be intercalated between the large myelinated ones; and that in other nerves such areas may be fairly free from non-myelinated fibers. Evidence presented previously shows that a group of fibers of characteristic distribution may pursue a separate course from the main trunk to join the vagus below the nodose ganglion. This group contains myelinated fibers which are among the largest in the nerve, small myelinated fibers, and a goodly number of non-myelinated. In this group practically all of the large and small myelinated and a large proportion of the non-myelinated degenerate following intra-cranial section of the nerve. We interpret this evidence to indicate that non-myelinated fibers of cerebral origin course in the cervical vagus in proximity to the large myelinated fibers destined for distribution through branches of the recurrent laryngeal nerve.

To eliminate the possible origin of non-myelinated fibers of the cervical vagus from the superior cervical ganglion, this ganglion was removed without injury to the nodose ganglion in each of three cats. In all cases an adequate time (20 days) was allowed for degeneration of interrupted fibers. We were unable to demonstrate in pyridine silver preparations of the cervical vagus any areas of degeneration whatever.

3. *The thoracic vagus and its branches.* Three experimental conditions designed to determine the origin of the fibers of the thoracic vagus were available for comparison with the normal: 1, section of the nerve above the nodose ganglion; 2, section below the ganglion; 3, removal of the stellate ganglion

Above the origin of the recurrent laryngeal nerve, the composition of the vagus trunk resembles that at the cervical level. As noted by others, the non-myelinated fibers appear to occupy a somewhat larger area than in the cervical vagus, but this may be due to the egress of myelinated fibers in the intervening stretch. Section of the nerve above the nodose ganglion produces only small loci of degeneration in the area of the nerve exclusive of that containing the recurrent laryngeal fibers. Even these are of inconstant occurrence. In the area occupied by recurrent laryngeal fibers a considerable area of degeneration is apparent. This may or may not contain a few normal fibers. Removal of the stellate ganglion does not have any appreciable effect upon the fiber content at this level. Section of the nerve below the nodose ganglion produces almost complete degeneration; a very few medium sized myelinated and non-myelinated fibers are observed scattered through the nerve; small groups of non-myelinated fibers are sometimes observed at the periphery. Similar findings have been reported by Chase (1916) in the dog.

The recurrent laryngeal nerve as it leaves the vagus is found to be made

up of two different groups of myelinated fibers (fig. 7, A). One of these, the large myelinated (7–13 microns) degenerates completely when the nerve is sectioned either above the nodose ganglion or intracranially. The other group consists essentially of small myelinated fibers (2–6 microns). It either forms a crescent at the periphery of the group of large myelinated fibers or leaves the vagus as a separate small nerve. Isolated fibers of the latter group are also scattered among the large myelinated fibers. The small myelinated group is not eliminated when the nerve is sectioned above the nodose ganglion (fig. 7, B), but is removed with the large myelinated fibers following section below the nodose ganglion. In the latter case, a

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Fig. 7. Photomicrographs, branches of the thoracic vagus, normal and experimental.

A. Normal recurrent laryngeal, pyridine silver. Note the division of the nerve into areas of large and of small fibers.

B. Recurrent laryngeal twenty days after section of the vagus nerve above the nodose ganglion, osmic acid. The large fibers have undergone degeneration, the field of small fibers appears intact.

C. Heart branch of the left vagus nine days following the removal of the stellate and upper thoracic ganglia. Note the size of the majority of the myelinated fibers as compared with those of the bronchial ramus at the same magnification, figure 7, G. Since extremely few myelinated fibers persist following cervical section of the nerve, we are justified in assuming that this photomicrograph represents the normal complement of myelinated fibers.

D. Heart branch after section of the nerve above the nodose ganglion, osmic acid. Whereas the myelinated fibers appear significantly less numerous than in C, indicating the loss of fibers with cells above the ganglion, a part of the difference may be due to variability between one preparation and another.

E. Heart branch, pyridine silver, showing that a significant number of small fibers, myelinated and non-myelinated, persist following section above the nodose ganglion.

F. Heart branch, pyridine silver. The large majority of its fibers were lost following section of the nerve below the nodose ganglion. Retouched to indicate in India ink the position of the persisting fibers.

G. Bronchial ramus, osmic acid, fourteen days after section of the nerve above the nodose ganglion. Note the persistence of the larger myelinated fibers. These and the smaller myelinated fibers probably are as numerous as in the corresponding normal rami.

H. Bronchial ramus, pyridine silver corresponding to G. Note the normal appearance of the branch.

I. Left vagus nerve below the level of the bronchial rami twenty days following section of the nerve above the nodose ganglion. K which was taken from a longitudinal section of the nerve at the same level demonstrates the normal appearance of the nerve.

J. Longitudinal section between the heart and bronchial rami twenty days following section above the nodose ganglion.

K. Longitudinal section below the bronchial rami twenty days following section of the nerve above the nodose ganglion. No alteration from the normal is apparent.

A and B 200 diameters; C, D, E, F, G, H and I 300 diameters; J and K 400 diameters. Zeiss 16 mm. Homal I.

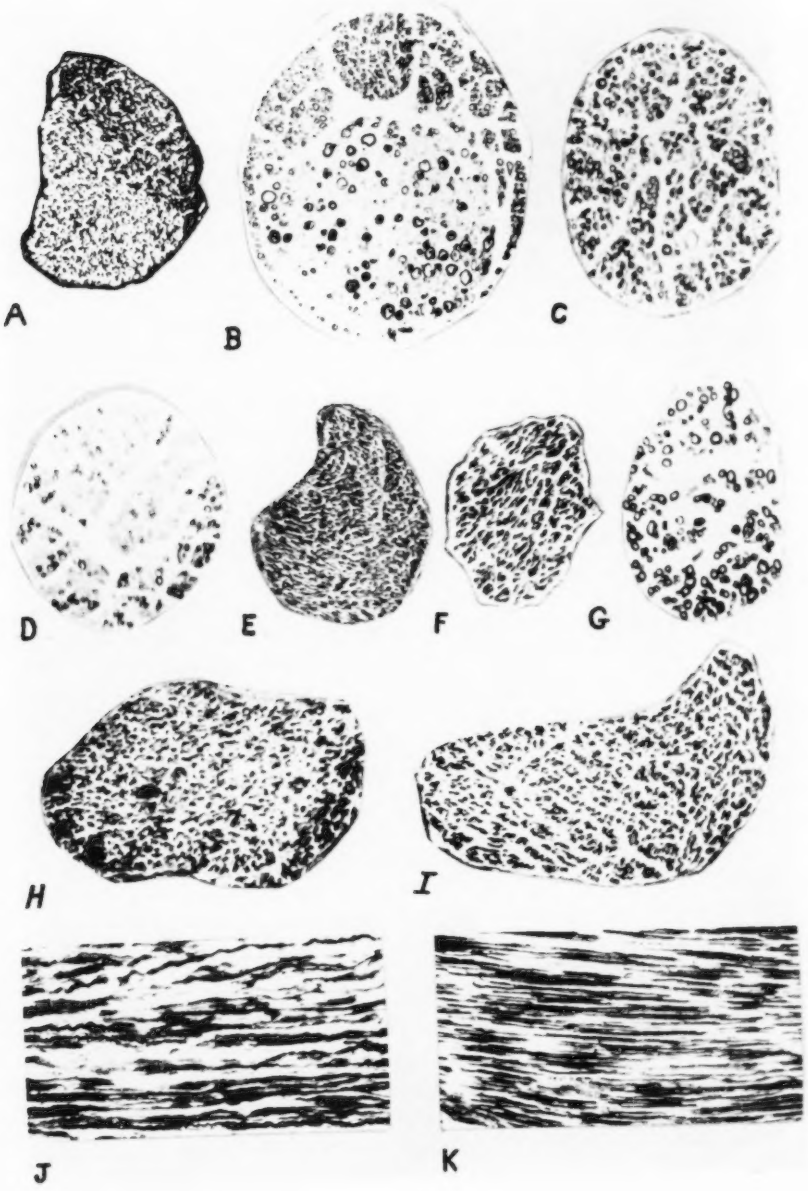

Fig. 7

small number of non-myelinated fibers sometimes persist. We can detect no difference in the fiber content of the recurrent laryngeal following removal of the stellate ganglion.

The heart branches of the vagus consist essentially of non-myelinated and small myelinated fibers 7 to 2 microns in size. Larger myelinated fibers, 10 to 7 microns, are relatively scarce. The content of 7 to 2 micron myelinated fibers is somewhat variable, but always significant; figure 7, C is taken from a heart branch (8 days after removal of the stellate ganglion) in which fibers of this size were somewhat more numerous than is usual. Other branches accompanying it had a lesser content of myelinated fibers. Studies of heart branches prepared by the osmic acid method twenty days following section of the nerve above the nodose ganglion indicate a small but appreciable loss of myelinated fibers as compared with normal heart branches (fig. 7, D). Removal of the stellate ganglion has no appreciable effect. Following cervical section of the vagus, never more than two or three myelinated fibers remain. In pyridine silver preparations evidence of degeneration is difficult to interpret except following cervical section. Then almost all fibers are removed (fig. 7, F); the few that remain can be accounted for by ganglion cells which occur within the branches. When the nerve is sectioned above the nodose ganglion or intracranially (fig. 7, E), a large number of non-myelinated fibers are found to persist. The lack of definite areas of degeneration does not permit a definite statement as to the number of fibers that are removed. Removal of the stellate ganglion does not have an appreciable effect.

The bronchial rami contain many of the 10 to 7 micron myelinated fibers, significantly fewer of the small myelinated ones which characterize the heart branches (fig. 7, G). The non-myelinated fibers appeared to be significantly less numerous, but the finding on one occasion of a pure non-myelinated branch attached to the mixed branches invariably found prevents a final statement on this point. In the dissection necessary to remove the branches, it is entirely possible that such a pure non-myelinated branch is frequently overlooked. The myelinated fibers show no perceptible reduction in numbers following section above the nodose ganglion (fig. 7, H); almost all are eliminated when the nerve is severed below the ganglion. The variability in the content of the bronchial rami in non-myelinated fibers mentioned above does not permit us to make any statement regarding the results of section above the nodose ganglion and removal of the stellate ganglion. However, groups may persist following section below the nodose ganglion, indicating that all non-myelinated fibers are not of vagal origin.

Below the level of the bronchial rami, the nerve contains almost exclusively non-myelinated (the large majority) and small myelinated fibers. Areas of degeneration can always be found following section of the nerve

below the nodose ganglion: the proportion of the total cross section which these occupy depends on whether or not an anastamosing branch from the normal vagus has joined the nerve. We are unable to identify an area of degeneration at this level following section above the nodose ganglion (fig. 7, I). Removal of the stellate ganglion similarly appears to have no effect. In each case a loss of non-myelinated fibers scattered throughout the nerve would not be apparent and the lower the section, the more liable would scattering of such fibers be to occur.

The lower abdominal divisions of the vagus have not been investigated, but it is obvious that the findings after degeneration in the thoracic trunk must in general apply; that is, that most of the fibers reaching the viscera of the abdominal cavity are non-myelinated, that most of these have their cells of origin in the nodose ganglion and that those, if any, which pass directly from the medulla with cells of origin central to the jugular ganglion are too few or too scattered to be detected by degeneration technic. The few myelinated fibers that are found at this level show the same distribution with non-myelinated fibers that seems to characterize motor autonomic fibers elsewhere, and the number of myelinated fibers of a size that corresponds to visceral afferents elsewhere (pain, vasomotor, etc.) is lower still. In view of the functional results reported above, it may be concluded that the vagus below the diaphragm is almost purely a motor nerve.

Discussion. It is evident from our experimental results that on a functional basis the fibers of the mammalian vagus nerve are divisible into two groups. One of these is composed of motor fibers to striated muscle and the entire complement of afferent fibers. These fibers, as in the turtle (Heinbecker, 1930, loc. cit.) have physiological properties similar to those of typical somatic fibers. All are myelinated and they comprise the largest fibers of the nerve trunk. The second group is composed entirely of autonomic motor fibers with physiological properties of a different order, four to five times slower than that for somatic fibers. These fibers are both myelinated and non-myelinated, the former having a lower threshold (ratio 1:10) and a faster conduction rate (1:10) than the latter, these ratios having reference to the fastest fibers of each group. Such a functional division of fibers does not correspond with the anatomical at the level of the nodose ganglion. Both anatomical groups, those coursing around the nodose ganglion, as well as the larger group coursing through the ganglion, possess myelinated and non-myelinated "autonomic" efferent fibers, the former containing, in addition, somatic motor fibers to the larynx.

The depressor nerve, regarded as a branch of the vagus, like the parent trunk, contains three fiber groups only the first of which is afferent (Heinbecker, 1932, loc. cit.). The fibers in this nerve with properties typical of "autonomic" efferent fibers presumably are concerned with the innervation of structures in the walls of the aorta, the muscle coats of the vasa

vasorum coming most strongly to mind. The locus of origin of the cells of these fibers is within the nodose ganglion.

The similarity of the vagus nerve to sympathetic and mixed somatic nerves is apparent. The sympathetic nerve trunks have been shown to contain afferent fibers with somatic nerve properties (Heinbecker, 1932, loc. cit.) as well as efferent fibers with the slower properties (Bishop and Heinbecker, 1932). In the saphenous nerve all the afferent fibers have somatic properties (Heinbecker, Bishop and O'Leary, 1933) and the slow fibers in its content are efferent (Bishop, Heinbecker and O'Leary, in press). The division of the autonomic nervous system into two systems, sympathetic and parasympathetic, does not rest on any fundamental difference in fiber type as does the division of the peripheral nervous system into somatic and autonomic. Such a division may be made arbitrarily on a basis of origin, craniosacral and thoracolumbar; but it does not indicate any fundamental difference other than the one of proximity to structures innervated.

On a basis of cell type the *motor* cells of the spinal ganglia with a central and a peripheral process are akin to those found in the nodose ganglion and not to those of sympathetic ganglia. However, as has been shown, not all vagus fibers have cells of this type, some having their cells of origin within the central nervous system. Fibers from cells of this latter type are also distributed to peripheral ganglion cells by the sacral roots. In the autonomic system, then, we have three types of fibers, those with cells within the central nervous system, those with peripheral ganglion cells having a central and peripheral process and those with peripheral gangion cells in synaptic relationship with preganglionic axons having their cells of origin within the central nervous system. The axons of the cells originating within the central nervous system, other than those designated as preganglionic, are concerned in whole or in part with the modification of the autochthonous activity of peripheral ganglion cells. Should the findings for the ganglion cells in the median nerve cord of the heart of Limulus polyphemus (Heinbecker, 1933) later be proved to hold for the visceral ganglion cells of the vertebrate then the influence of such fibers is not exercised through a one to one pre- to post-ganglionic relationship characteristic in sympathetic ganglia as shown by Heinbecker (1930, loc. cit.) for the turtle and by Bishop and Heinbecker (1932, loc. cit.) for the cat, but rather as a positive or negative "trophic" or "tonic" influence. In the autonomic ganglion cells with central and peripheral processes no evidence of autochthonous activity has been discerned. In this respect, then, they are similar to the ganglion cells of the paravertebral sympathetic system.

Functionally, the parasympathetic system is frequently antagonistic to the sympathetic, but such antagonism of action cannot now be regarded as invariably indicating the presence of fibers of both systems, as in our experimental results evidence of the presence of fibers of undoubted vagus

origin exerting both excitatory and inhibitory influences on the duodenum was obtained. This subject will be dealt with more fully in a subsequent publication. Histologically, our studies, as well as those of Jones (1932) indicate an increase in the number of non-myelinated fibers post-ganglionically to the nodose ganglion. If this be the case, it is necessary for an explanation to assume either early branching of peripheral axons or more than one peripheral axon for each ganglion cell. The function served is undoubtedly one of multiplying the channels of distribution. A similar purpose is accomplished in the peripheral sympathetic ganglia by having one pre-ganglionic fiber synapse with more than one ganglion cell (Billingsley and Ranson, 1918). Branching of motor fibers in the somatic system has been demonstrated by Eccles and Sherrington (1930).

The histological studies of the vagus branches to the heart reveal an interesting feature, namely, the persistence of many small myelinated and non-myelinated fibers after removal of the stellate and upper thoracic ganglia and after cutting the nerve above the nodose ganglion. The persistence of myelinated fibers is readily explained since those which remain are essentially of somatic type and for them an afferent function has been determined. Many of them are of the depressor and pain fiber size. Definite evidence indicating the function of the non-myelinated fibers which persist is lacking. No afferent function has been demonstrated. Undoubtedly they have their cells of origin in the nodose ganglion since but few fibers remain in the heart branches when the vagus nerve is severed in the neck. Fibers of similar type with cells of origin in the spinal ganglia are vaso-dilators; consequently, the fiber distribution to the blood vessels of the heart after section above the nodose ganglion is being investigated.

SUMMARY

The mammalian vagus nerve contains four functional fiber groups, somatic motor and somatic sensory fibers, autonomic myelinated and non-myelinated fibers.

The potential recorded from the cervical vagus after conduction shows three major potential maxima, the first derived from the somatic fibers, the second and third from the autonomic fibers.

The motor fibers to the larynx and fibers modifying the inotropic and chronotropic activity of the heart are derived from cells located within the central nervous system. Non-myelinated fibers whose activity results in constriction of the bronchi and in excitatory and inhibitory effects on the intestine (duodenum) arise from cells located within the nodose ganglion.

No evidence that fibers synapse in the nodose ganglion has been secured. It follows, then, that motor cells with a central and peripheral process exist in the nodose ganglion as well as cells of this type with afferent functions.

All afferent functions served by the vagus nerve are mediated by myeli-

nated fibers of the somatic type. The non-myelinated fibers serve no recognizable afferent function.

Afferent fibers from the lung modifying respiration are larger than those modifying the activity of the vaso-motor center. Pressure applied to the vagus nerve permits a differential blocking of these two afferent fiber groups, the former before the latter.

The vagus nerve at the level of the nodose ganglion shows two anatomical divisions, one containing the motor fibers from the larynx and autonomic fibers to the heart, the other motor and sensory fibers to the lungs and abdominal viscera.

Non-myelinated fibers with cells of origin in the nodose ganglion are also found in vagus heart branches.

The cervical sympathetic contributes few, if any, non-myelinated fibers to the cervical vagus nerve.

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THE FUNCTION OF THE NON-MYELINATED FIBERS OF THE DORSAL ROOTS

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We propose in this paper to discuss the groupings of fibers which appear to have a functional significance in peripheral nerves, and in particular, the functions of the group of non-myelinated fibers in the dorsal roots of mammalian nerves. We will consider first, the evidence that a correlation is possible between the histological structure, the physiological properties and the functional relations in the body of the various groups of fibers; second, the differentiation by means of pressure block of the activities of the dorsal root non-myelinated fibers, from those of all others in the saphenous nerve, and our failure to find any afferent function for such fibers; and third, evidence for a *reflexogenic motor* function of vasodilatation assignable to these fibers, similar in all respects to that which has been inferred to be antidromic activity.

The activity of non-myelinated fibers is detected by means of the cathode ray oscillograph and amplifier, in terms of the appearance of the so-called "C" wave, propagating at about one meter per second under experimental conditions in mammalian nerve at 38°C. This correspondence between the C wave and non-myelinated fibers has been questioned (Erlanger and Gasser, 1930) and, we believe, answered (Bishop and Heinbecker, 1930). Even if it were not true however that this wave is exclusively due to fibers which fail to stain in osmic acid, and which stain more or less differentially in pyridine silver, our present results would still mean that there are "C" fibers, (character in question), passing into the cord via the dorsal roots, with their cells of origin in the dorsal root ganglion, for which we can find no afferent function, and for which a reflex motor vasodilator function is indicated by further experiments. But obviously it is desirable to know specifically which fibers are being dealt with; and this being known, the non-specific designation "C" becomes merely a convenient abbreviation for a specific group of properties. This is more to the point since Blair and Erlanger recently (1933) "fail to support" our classification of nerve fibers into recognizable types.[1] The evidence that non-myelinated

[1] Pending full publication of their results, it is not critical for our present investigation whether fibers of a nerve fall into types which are quite distinct from each

fibers can be recognized physiologically has been published as it accumulated over a period of years, and will be briefly summarized here.

1. *The properties of non-myelinated fibers, or the histology of the "C" wave.* Heinbecker (1929) first reported on the properties of non-myelinated fibers in the turtle and cat sympathetic and vagus nerves, as conducting at 1 meter per second or less, with a long duration of action potential, chronaxie 3.5 to 6 sigma, threshold of 50 to 100 times that of the most irritable fibers, and refractory period of 4 to 6 sigma. They did not follow the fiber size rule found by Gasser and Erlanger (1927) to apply to faster fibers. Fibers with similar conduction rates were found by Erlanger and Gasser (1930) to enter spinal nerves from their dorsal roots, and since then practically every nerve analyzed has been found to contain this component either of spinal or of autonomic origin, often from both. The fibers have practically identical properties in sympathetic, parasympathetic and dorsal root systems, and in all cases where concrete proof is available, they are found to be motor in function. Heinbecker (1930) observed that the relative magnitude of this potential corresponded with the relative number of non-myelinated fibers in various nerves, and Bishop and Heinbecker (1930) found certain nerves, for instance intestinal branches of the coeliac plexus where the predominance of two types of fibers, large myelinated and non-myelinated (the action potentials of the former being definitely recognizable), left no room for doubt that the non-myelinated fibers of these nerves produced the C waves; 10 per cent of the cross sectional area of a nerve occupied by myelinated fibers corresponded to 7 per cent of the total time-potential area occupied by the faster of the two waves. In the cervical sympathetic of the rabbit (Bishop and Heinbecker, 1932) a more striking situation exists. Practically all the post-ganglionic fibers above the superior ganglion are non-myelinated, (there are a few afferent fibers of cranial origin), and only a C wave appears beyond the ganglion when the preganglionic nerve is stimulated and only a C threshold is found post-ganglionically for sympathetic motor effects. Pre-ganglionically, the fibers are mostly small myelinated ones, and the threshold for the same motor effects is correspondingly lower, with conduction rate and other properties to correspond. The cervical sympathetic branch to the heart of the monkey (unpublished) gives only a small B and a larger C wave, and the fibers are almost exclusively non-myelinated. The vagus of cat and rabbit gives a large C wave, and in the lower region along the stomach where almost all the fibers except non-myelinated ones have left in branches, the C wave

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other, or whether they fall into certain prominent and recognizable groups, without absolute discontinuities between them. In the saphenous nerves of cat, rabbit, dog and man, there are no fibers detectable with rates of conduction between 6 and 2 meters per second, which leaves the group of fibers in question satisfactorily isolated for a study, a discontinuity existing even in conduction rates.

becomes a correspondingly larger part of the total potential. On the other hand, the cervical sympathetic which is without significant branching gives a small but constant height of C wave, throughout its length, corresponding to the low proportion of non-myelinated fibers.

In the saphenous, to be dealt with below, a similar correspondence can be inferred. The properties of the C components from sympathetic rami and from the dorsal roots are indistinguishable. After section of the dorsal roots supplying the saphenous of the cat, (Heinbecker, O'Leary and Bishop, 1933) the sympathetic component remaining consists exclusively of non-myelinated fibers, and the only potential elicited upon stimulation of the nerve is a C potential.

By quite similar differential procedures, on nerves selected because of their suitable fiber constitution, a second slowly conducting component of potential (15 to 3 m.p.s. in mammals) has been assigned to small myelinated motor fibers of the autonomic system; a faster sensory component has been assigned to small myelinated fibers of similar appearance histologically to the autonomic, but with different physiological properties. These two components have been assumed by Erlanger and Gasser (1930) to be homologous, and to be motor post-ganglionic, and have been designated by them the "B" wave. We have referred to them as the B_1 and B_2 divisions, recognizing that the B_1 or sensory component is in all essential respects more like the A group of faster myelinated fibers than like the second or B_2 component, and that no discontinuity exists in the A and B_1 properties, until we come to autonomic fibers of the B_2 group.

As for the immediate background of the present investigation, it has been found that the groupings of fibers which cause separate waves to appear in the total action potential record have a significance in relation to the normal functioning of nerve fibers in the body. In general, the fibers in any one such group tend to serve a common function, and in many cases a given function is mediated *only* by such a circumscribed group of fibers in a given nerve. Vagus effects on the heart (Heinbecker, 1931), various functions mediated by the cervical sympathetic (Bishop and Heinbecker, 1932) and in the saphenous, mediation of pain and touch by separate groups (Heinbecker, Bishop and O'Leary, 1933), may serve as examples, of which other instances are found, both by ourselves and by others (Erlanger, 1927). In the saphenous, certain typical sensory effects have been found to be associated with each group of fibers recognizable by their action potentials, except the non-myelinated group, although such individual groups may at the same time serve more than one function. This non-myelinated group is the one whose stimulation in the sciatic nerve causes vasodilatation of the limb and the Sherrington pseudomotor contracture.[2] (Hinsey and Gasser,

[2] Hinsey and Gasser identified these fibers as "C" fibers. Their identity with non-myelinated fibers is inferred by ourselves from such correlations as have been noted above.

1930.) In studying the sensory effects of stimulating superficial sensory nerves in animals and man, (Heinbecker, Bishop and O'Leary, loc. cit.) sensory effects from stimulation of these fibers could not be satisfactorily evaluated because of their high threshold, which required that all other fibers in the nerve be activated at a stimulus strength that would activate these, and since intense pain was caused by stimulation of fibers of lower threshold, this masked any possible further effect. All we could say was that no sensory or reflex effect of stimulation of these fibers was *detectable*

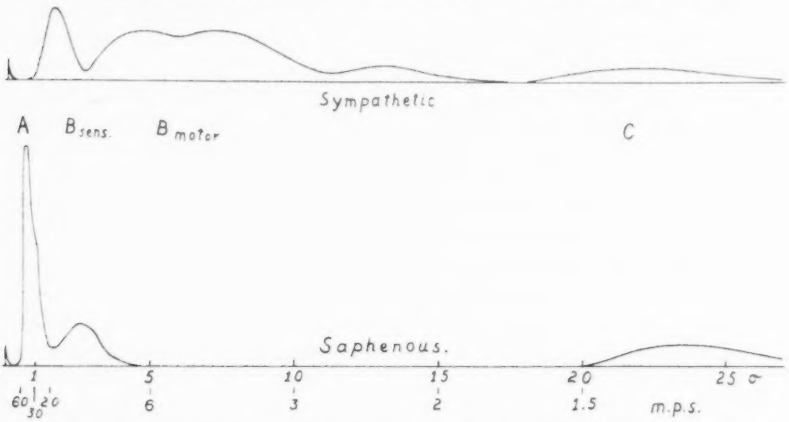

Fig. 1. Diagrammatic representation of potentials after conduction in the cervical sympathetic (upper) and saphenous (lower) nerves of the cat. The rabbit differs only in the lack of the afferent first wave of the sympathetic record, which then occurs in the separate depressor nerve. Note first, that taking both nerves together, there is no range of conduction rates where activity is lacking, and the division of the potentials of a particular nerve into A, B, and C groups on this basis has not a general significance; for instance the gap between A and B in the saphenous is filled by the first wave of the sympathetic (depressor). Note, second, that in the saphenous, in the range of 6 to 2 m.p.s. conduction rate, no fibers are represented, while this range is occupied in other nerves containing autonomic B_2 components. The autonomic and the dorsal root C components of the saphenous both fall in the same range, which is the same as that of the autonomic C of the cervical sympathetic. The total area of the C potential of the saphenous is larger than that of the B, a longer duration compensating for a lower amplitude.

in the presence of that resulting from stimulating all fibers of lower threshold. We have now been able, by means of pressure block, to completely eliminate the sensory effects of all fibers of lower threshold than that of the C wave, leaving the conduction of impulses intact in from 75 to 100 per cent of the non-myelinated fibers. In such cases, while only a slight release of pressure, allowing a few fibers of faster conduction to take effect centrally, results in a violent response of the animal indicative of pain, no afferent

effect whatever can be detected from the stimulation of *all* the non-myelinated fibers at an even higher frequency, when fibers of lower threshold are blocked by pressure.

2. *Tests for afferent function in dorsal root non-myelinated fibers.* The saphenous nerve, except for a small sympathetic component of non-myelinated fibers, is entirely of dorsal root origin in the cat (Heinbecker, O'Leary and Bishop, 1933). The larger myelinated fibers enter the dorsal columns of the cord, the smaller myelinated and non-myelinated enter the tract of Lissauer (Ranson, 1913). The action potential after conduction separates out into three elevations, the latter two being separated by a distinct gap, which in many other nerves is occupied by the potential of autonomic myelinated fibers (fig. 1). The interval in this region makes it possible to obtain, by means of differential pressure block, a clean separation of the last two elevations, a result which is not possible in nerves where the intervals between groups are less; that is, where this particular interval is occupied by autonomic fibers, as it is in the cervical sympathetic or vagus nerves, or in the sciatic of the frog. In other words, while pressure tends to act differentially on different groups of fibers, depressing the most irritable fibers most severely (Gasser and Erlanger, 1929) complete separation of closely adjacent groups by this means is not possible. In the cat (2 experiments) we could not separate completely even the last two groups in the saphenous, although the conduction rate interval between these groups in the cat is quite as definite as in the dog or rabbit, where such separation is readily obtained.[3]

[3] There are only two agencies so far investigated which affect the faster fibers of a nerve before the slower, galvanic polarization and pressure. Polarization by galvanic current seems to be a little less sharply differential than pressure. What Lewis, Pickering and Rothschild (1931) believe to be the effect of ischemia and which works in the same order, we assign to the pressure by which the ischemia was produced, for two reasons. In the first place, asphyxia, like most other depressing agents, including lactic acid and CO_2 (Heinbecker, 1929; Heinbecker and Bishop, 1931) depresses the slow fibers first. Secondly, Lewis, Pickering and Rothschild seem to infer from Gasser's work, that only the relatively high pressures employed by him will block nerves. Lower pressures require an inconveniently longer time, and Lewis, Pickering and Rothschild's pressures did not block sympathetic fibers even after ½ hour. The following experiment we believe indicates the state of affairs. If pressure is applied to a nerve or to a limb with the nerve in situ, sufficient to block the fastest group of fibers in, say, one minute, the first rapid decrease of potential, as observed on the oscillograph after conduction past the block is followed by a slower decrease that falls very gradually to a lower level. This first pressure will be sufficiently high to occlude all vessels, but not all fibers are blocked for an indefinite time. A further increment of pressure in completely ischemic nerve, will now cause a second sudden decrease of potential and moreover, of a potential that would not have been blocked at all by the first, followed by a more gradual fall to a second level; and the third increment will repeat the effects of the first two. There is no reason for believing that the second and third effects which are obviously due to pressure in nerve already ischemic are in any way different from the first.

The procedure was as follows. In an animal (dog or rabbit) anesthetized by ether the saphenous nerve was dissected free through an incision in the skin, up to the knee, then a length of at least 2 cm. was left in situ and 3 cm. exposed again along the femoral artery high up in the groin (fig. 2). The free peripheral end (3 or 4 cm.) was drawn onto electrodes, covered with a glass shield, for stimulation, and the whole apparatus strapped to the limb and wrapped in towels for warmth. A single lead electrode was hooked under the upper stretch of nerve, and connected to the amplifier grid. Either the limb itself was connected to ground, or a divided ground electrode made contact with the nerve on either side the grid electrode, giving in either case a triphasic lead from the nerve without interfering with conduction of impulses past it centrally. Between these two sets of elec-

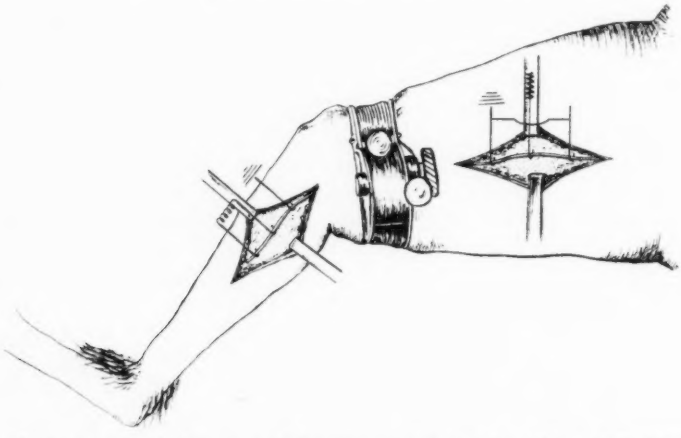

Fig. 2. Arrangement of nerve and of pressure apparatus for differentially blocking impulses in certain fibers of the saphenous nerve. See text.

trodes, pressure was applied either by means of a parallel jawed clamp pinching the tissue around the undissected nerve, or by a leather thong about the whole limb, tightened with a reel. The animals were allowed to recover from ether until stimulation of the unblocked nerve caused whining and struggling and respiratory effects (the rabbits made no vocal protest), and blood pressure was in most cases recorded from the opposite femoral artery. Stimulation frequency was varied from 2 to 20 per second, the nerve response was recorded by means of the cathode ray oscillograph.

It has previously been found that no stimulation of superficial nerves in animals or man up to 60 per second causes pain, unless the strength of stimulus is sufficient to activate the B wave (Heinbecker, Bishop and O'Leary, l.c.). Stimuli activating one-third of this wave at 2 per second

or even single stimuli involving more fibers, cause unmistakable pain, the sensation increasing both with the frequency and with the number of fibers stimulated up to the full extent of this wave. Blood pressure and respiratory effects and reflex and, in humans at least, purposeful responses go parallel. Now when pressure was applied to the nerve in animals, all these results of stimulation fell off in proportion as the fibers giving this wave were blocked, even though the non-myelinated fibers producing the C wave were responding maximally to each stimulus. There was never any decrease in the area of this last wave until the preceding one was half blocked, usually the C wave was not more than 10 to 20 per cent decreased in height when the B wave had completely vanished, and in several cases the C wave had been maximal for a time sufficient for ample tests, after the B wave was gone. In no case was there any reflex activity whatever that could be assigned to the responses of non-myelinated fibers, and the amount of such activity was in all cases roughly proportional to the area of the B wave. The results were reversible if pressure was not kept on too long at a time, and the cycle could be repeated several times in each animal before failure of the nerve, each application of and recovery from pressure requiring about $\frac{1}{2}$ hour.

While the above statement of results is literally correct, it would not be proper to leave it without a word as to the mechanical and physical complications of stimulating non-myelinated fibers in vivo. The threshold for the most irritable of these fibers is about 50 times that of the most irritable fibers (touch fibers) in the nerve, and five or ten times that of the most irritable pain fibers. In the adjacent tissue moreover there are pain *endings* that can easily be stimulated by spread of current. In maximal stimulations of the non-myelinated fibers, a stimulus at least three times threshold for these fibers is required. Furthermore, this term stimulus strength applies to battery voltage and not to quantity of current, and a longer duration of shock is required for the non-myelinated fibers. For instance, a nerve in which the threshold for touch fibers is 1 volt from a 0.0005 m.f. condenser, no C appearing even at 200 volts, will require 50 volts from a 0.1 m.f. for threshold of the C wave and 150 volts for its maximum. The ratio in quantity of current is thus 1:3000. Such quantities of current require considerable caution lest spurious effects be obtained by leakage past the point of block. To enable one to observe the B wave precisely when stimulating the C maximally, with a sensitivity of apparatus adequate to observe both, (400 mm/mv) it has been found necessary to put the stimulating electrodes in one arm of a Wheatstone bridge and balance against a fixed resistance, and one point of this bridge is grounded. A second ground is placed on the nerve between stimulation electrodes and the body, to further minimize the stimulus distortion. One of the leads is also grounded however, and this forms a possible pathway for current from the stimulat-

ing circuit past the point of block. Response to such a stimulus will follow the shock disturbance so closely at the lead electrodes that it cannot be distinguished from this, and of course will have a central effect no matter how perfectly the pressure block has interrupted the pathway peripherally to it.

Spurious effects of this kind have been encountered in some of our experiments, suggesting activity assignable to non-myelinated fibers. In every case met with, more careful adjustments of the circuit eliminated them, without any obvious change in the general state of the animal. The result we wish to emphasize is that in every experiment a C wave could be conducted centrally without reflex effect, under conditions in which a slight reduction of pressure, with appearance past the block of only a part of the B wave, produced marked effects on respiration, blood pressure, or muscular activity, and obviously, upon painful sensation. The group of fibers of which the pain fibers are typical is obviously the predominantly reflexogenic one in the saphenous, to the virtual exclusion of other groups under our conditions.[4] The group includes fibers for temperature (Heinbecker, Bishop and O'Leary, l.c.).

3. *Reflexogenic vasodilatation via dorsal root non-myelinated fibers.* Lacking an afferent function assignable to the fibers under discussion, our obvious recourse was to test whether the vasodilatation known to occur via the dorsal roots, and conventionally termed "antidromic" (Bayliss, 1901), normally followed sensory stimulation of afferent endings through an axon reflex, or whether there was really a motor function assignable to these fibers, reflexly activated via the spinal cord. Since vasodilatation due to depressor nerve stimulation in the rabbit is pronounced and reproducible, we have employed this reflex, on the possibility that so large a depression of blood

[4] This statement requires several qualifications when applied to other than these experimental conditions, and it presumably does not apply to all nerves other than the saphenous. The A group contains fibers whose stimulation causes some changes of blood pressure but apparently no respiratory or muscle motor effect; and a striking and abrupt increase of effect on blood pressure comes in with the B wave. There are fibers present here for both pressor and depressor effects (we believe, different fibers, but with similar thresholds), and it is not clear whether such fibers are identical with pain fibers, or separate. The predominance of pressor over depressor effects depends on the state of the animal and upon the frequency of stimulation, and the commonest response is a fall of pressure followed by a rise, at rates below 20 per second. The proprioceptive sensory fibers from muscle may not be confined to the B group, but these do not run in the saphenous and pronounced reflex movement is only elicited from this nerve above the A maximum. In a few decerebrate animals tested, results were not essentially different. The cremasteric reflex in the male dog is confined to the B range in the saphenous. In humans, beautiful reflex responses are elicited in the B range from stimulation of superficial skin nerves of the arm, but not below this range (Heinbecker, Bishop and O'Leary, l. c.). None of these qualifications compromise the statement that non-myelinated fiber responses have no reflex effects under conditions where stimulation of fibers in the pain range has pronounced effects.

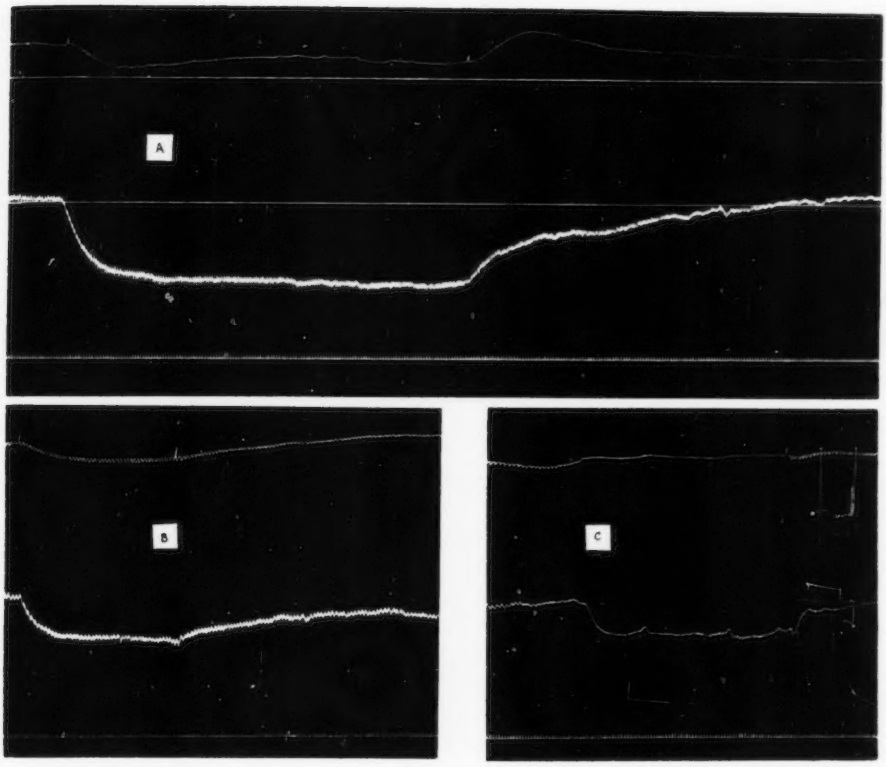

Fig. 3. Plethysmographic records of volume changes in the hind limbs of rabbits. Reduced to ½ size. Time in seconds. Time record is zero line for blood pressure.

A. Sympathectomized limb, dorsal roots intact, depressor nerve stimulated. A passive fall of volume accompanied a fall of systemic blood pressure, followed by a rise during constant blood pressure. A passive rise of volume on rise of blood pressure at end of stimulation is superposed upon the previous rise, the sum of these two being greater than the normal before or after the stimulation. Note the delayed rise of volume, and the delayed fall after stimulation. Time of stimulation marked on plethysmographic record at top.

B. As above, but completely denervated limb. Change of volume of the limb follows the systemic blood pressure passively.

C. As above, but normal rabbit, and vagus stimulated, with a pure depressor effect an unusual result. There is no significant change in volume of the limb, relaxation here just compensating for the effect of reduced systemic pressure. There may, in some cases, be a slight rise in volume of the normal limb on depressor nerve stimulation.

pressure (over 50 per cent at maximum) would require all the means at the animal's disposal for its accomplishment. The problem was, first, to eliminate the decrease of tone of the sympathetics, as a factor in dilatation, and, second, to obtain a reliable index of vasodilatation itself in an animal as nearly normal as possible.

We have recorded the volume of the limb by means of a plethysmograph, and the flow of Tyrode solution perfused through the femoral artery, in animals under light ether anesthesia, with one limb previously sympathectomized, the normal or the completely denervated limb used for control. In cats and dogs the vagus was stimulated, after section of both vagi peripherally. In rabbits the vagus and depressor were dissected out together and the vagus was cut centrally to the dissection as well as peripherally; this furnished a larger nerve mass which dried less readily and could be handled with safety. The opposite vagus was cut. Maximal stimuli were usually applied, at 5 to 10 per second, the nerve lying across metal electrodes in air, with no precaution as to temperature in a room at 25°C. Drying was the only complication which arose, and was dealt with by means of an atomizer filled with Tyrode's at room temperature.

Reflex increase in volume of the sympathectomized limb. The leg to be tested was drawn into a water-jacketed plethysmograph, and sealed about the thigh with a rubber membrane daubed with vaseline, and temperature equilibrium attained. A bellows recorder with water seal wrote changes of volume on the kymograph. Anesthesia just sufficient to produce quiescence reduced the blood pressure but slightly, the usual figure being 110 mm. Hg in rabbits. Typical records for the sympathectomized and normal limbs appear in figure 3.

With a drop of systemic blood pressure up to 50 per cent, a *passive* decrease of volume in even the sympathectomized limb might be expected. This is what in fact occurs initially upon depressor nerve stimulation. In the completely denervated limb the fall is still greater; and in the normal limb the volume may increase a little. In other words, in the normal, the reduction of systemic pressure (due also to visceral dilatation) just about compensates for relaxation (not dilatation) of limb vessels, and the total limb volume may be little altered.

However, in the sympathectomized limb, the initial, presumably passive, fall of volume is followed by a rise, to normal or slightly above, with no return of systemic pressure, and sometimes while the latter is still falling. At the end of stimulation this relative rise becomes an absolute one, which persists while the systemic pressure is returning to normal, but usually falls off before complete recovery of the latter. In other words, there is a lag of several seconds in the dilator mechanism of the sympathectomized limb, behind that of the system as a whole, both in onset and in recovery. In most of our curves in fact the systemic pressure falls by two stages, the first

rapid fall sometimes levelling out before the second more gradual fall is in evidence. In this case the reversal of the decrease in volume of the limb to a relative increase coincides with this second stage. The first stage obviously involves inhibition of the sympathetic. The second perhaps connotes activation of the dorsal root dilator mechanism, both in the sympathectomized limb and in the system as a whole. The delay noted is of the same order as the latent period observed upon direct stimulation of the dorsal roots, indicating a latent period at the periphery.

Passive decrease in volume of a completely denervated limb with fall of systemic pressure is to be expected if no other innervation involving blood vessels were functioning. On the other hand, the difference between the sympathectomized and denervated limb is not striking; the two limbs were not recorded coincidentally, and the fact that in the normal limb relaxation of the vessels only approximately compensates for fall of pressure emphasizes the complication that the varying systemic pressure introduces. Total sympathectomy has been shown by Freeman and Rosenblueth (1931) to still permit a reflex fall of systemic pressure by stimulation of the depressor of the rabbit, and our results fall in with this. On the other hand, we have not consistently obtained, even in dogs, the marked rise of volume of the sympathectomized limb that Fofanow and Tschalussow (1921) reported to follow stimulation of the vago-depressor trunk of these animals. (For results of stimulation of the vagus, see below.) We therefore hesitate to draw the obvious conclusion from these results alone that dorsal root vasodilator fibers were reflexly activated, but present the experiements for what they are worth, and pass to a differential perfusion procedure.

Perfusion of the sympathectomized limb. A number of unilaterally sympathectomized animals were perfused through the femoral artery of the operated and normal limbs separately, rate of flow being recorded before and during stimulation of the depressor. The perfusate was allowed to return through the veins to the systemic circulation. Increases of flow were obtained, greater increases of course through the normal than through the sympathectomized limb. Although the perfusing pressure was held constant, the systemic blood pressure naturally fell during stimulation, and this raised the question whether altered venous pressure or altered pressure on the circulation collateral to the femoral system could be disturbing factors. In the completely denervated limb, a small increase of perfusion flow accompanied lowering of blood pressure; when the denervation was accomplished by low spinal section the increase was very slight. The increase of venous back pressure in the limb consequent upon peripheral dilatation elsewhere should cause a decrease of flow. The reduction of pressure on capillary or arterial collaterals therefore more than compensates for this. The venous pressure would be so small a fraction of the total perfusion pressure that such a result might be expected. The col-

lateral circulation however cannot be disregarded, especially if there were any sympathetics intact to vessels supplying the pelvis.

Assuming that in the sympathectomized limb the caliber of collateral vessels remained constant during a lowering of blood pressure, the flow through them would depend on the difference between perfusing and systemic pressures. If the former were higher, a decrease of the latter would permit greater flow from perfusing system into the circulation. If the perfusion pressure were lower than the systemic, the amount of blood entering the perfusion system would decrease, when the systemic pressure fell, allowing again an increase of the flow from the perfusing source. Even though the actual pressure on collateral vessels were less than the perfusing head, as it would be in general, this should make no difference in the above argument. Either situation would imitate dilatation of the limb, with no criterion for distinguishing between active and passive increases of flow into it. We therefore undertook to balance the effects of collateral circulation in the two limbs against each other, thereby recording the immediate effects of nerve stimulation in only one of them.

The most significant result of the preceding series was that in the denervated limb a slight increase, and in the normal a large increase of perfusion flow followed immediately upon stimulation, parallel to the fall of systemic blood pressure; while in the sympathectomized limb a further delayed increase took place, reminiscent of the delayed increase of volume in the plethysmographed limb.

Differential perfusion of the denervated and sympathectomized limbs. The two hind limbs of rabbits were perfused in parallel from a common supply of Tyrode solution with 0.2 per cent glucose (Bayliss' formula), through cannulae in the femoral arteries inserted half way between knee and groin, in a stretch without conspicuous branches. Ether was administered, and the depressor stimulated, as before. Perfusion pressures equal to and below normal arterial pressures were used. An attempt was made to cannulate the veins, but mixing of the perfusion fluid with blood from collateral circulation and with tissue fluids (usually a light pink color showed in the outflow) was sufficient to cause clotting at the outlet, and it was found that the animals, especially rabbits, could take care of the perfusate for a time sufficient to record significant results if this was allowed to flow back through the veins into the systemic circulation. Needless to say, profuse urination occurred, and the blood pressure rose gradually from 110 to about 130 mm. Hg by the end of a series of readings, and considerable edema developed, without however any serious interference with perfusion, if experiments were performed expeditiously.

The auxiliary apparatus consisted of a fluid "Wheatstone bridge," as shown in figure 4. From a reservoir *A*, fluid passed to coils immersed, one in hot, one in cold water, *B*. The cold line was only employed to investigate the

temperature effect in perfusion, which proved to be insignificant over the range of our variations; in routine experiments fluid was used whose temperature as it passed the manometer was 30 to 35°C. The fluid dropped through a flask of oxygen, from the bottom of which it passed through a cotton plug filter. The divided flow passed two screw pinch cocks which

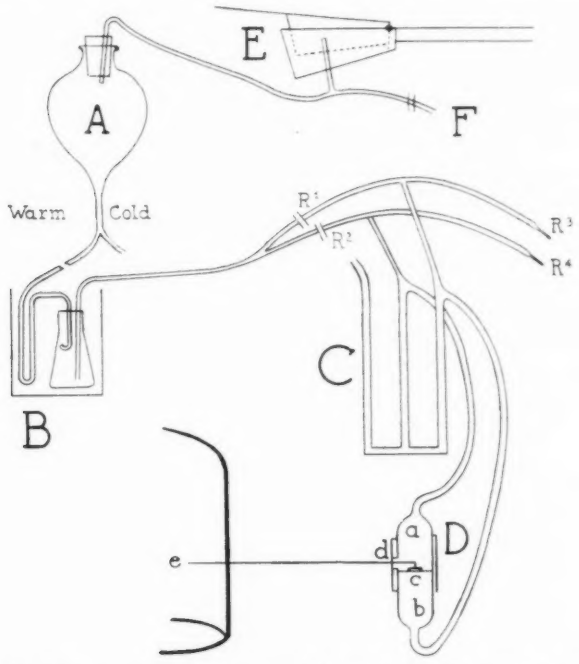

Fig. 4. Perfusion apparatus. *A*, pressure bottle feeding by gravity into *B*, warm bath containing flask of oxygen; thence to two pinch cocks R_1 and R_2 which with the two cannulae in the rabbit's femoral arteries, R_3 and R_4, form a fluid Wheatstone bridge. *C* is a differential mercury manometer, across the bridge as a detector of balance, and *D*, a differential membrane manometer whose pointer writes upon the kymograph at *e*; *a* and *b*, glass tubes, *c* and *d*, thin rubber diaphragms. *E*, bellows recorder which measures the volume of air flowing into *A* to displace perfusing fluid, when tube *F* is clamped. For further details see text.

could be accurately regulated as two arms of the bridge (R_1 and R_2). The animal's two limbs to which the cannulae led (R_3 and R_4) comprised the other two arms. Between R_1 and R_3 and between R_2 and R_4 were connected tubes to the differential mercury manometer *C*, whose common limb open to the air permitted the measurement of the actual perfusion pressure on the cannulae, and whose two differential limbs showed the difference of

pressure, that is, the balance of the bridge. In parallel with these two manometer arms was connected the recording manometer *D*. This was constructed of two glass tubes *a* and *b* 2 cm. in diameter, over one of which was tied a thin rubber diaphragm *c*, and the two tubes were connected by a heavy rubber tubing. Through the latter a small hole was cut at *d*, and this was covered with thin rubber. A lever, attached to the middle of the transverse membrane, penetrated the membrane over this side opening, and with this as a fulcrum, wrote the movements of the transverse membrane on the drum at *e*. The apparatus was extremely sensitive, the pointer deflecting to a difference of pressure too small to be read on the mercury manometer.

In use, the bridge was balanced only before nerve stimulation was to be applied, by adjusting R_1 and R_2. During the test, the deflection of the lever followed the change in pressure occasioned by the difference of flow

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Fig. 5. Records of time in seconds (bottom); membrane manometer deflection indicating differential perfusion flow; systemic blood pressure from carotid; rate of flow of air displacing perfusion fluid in reservoir (in record B); and time of stimulation, recorded as a notch on the upper record.

A. Both limbs sympathectomized previously, right limb denervated at time of experiment. A rise in the manometer record indicates an increased flow through the sympathectomized limb relative to that through the denervated limb. Note characteristic delay in response, the immediate passive effect of systemic fall of pressure on both limbs being compensated by the bridge circuit. Following the depressor nerve stimulation period, a second fall of blood pressure, apparently spontaneous and not accompanied by bodily movement, was followed again after a delay by an increase in perfusion flow through the limb with intact dorsal root innervation.

B. Experiment similar to above, delay both in onset and cessation of perfusion flow increase through sympathectomized limb, as compared to fall and recovery of systemic pressure. Increased flow is recorded as a changed slope of the manometer curve rather than as displacement (see note 4).

C. In the same animal as B with bridge slightly off balance, the perfusion pressure bottle was raised and lowered to cause pressure change about equal to blood pressure changes recorded in B. Note that the change in the bridge balance is recorded immediately causing an angle in the record. Compare delay in B.

D. With bridge balanced, a slight change of one pinch clamp (one arm of the bridge) causes an immediate change of direction in the curve in contrast to the delayed change following blood pressure lowering by nerve stimulation.

Note in records A and B (see also fig. 3, A) that the blood pressure falls in two stages, the first immediately after stimulation is begun, and the second, much less in degree, accompanying the change in perfusion flow. The latter is not visible in all records.

Record A was repeated six times in the same preparation, the effect falling off as the rabbit became edematous. This was the most striking of four experiments with differential perfusion. Two gave results of the magnitude represented in B, and one showed a still smaller effect, with none in the opposite direction. The effect was reduced by anesthesia more than just enough to produce quiescence, and reduced or abolished at the stage when anesthesia (by ether) began to lower blood pressure

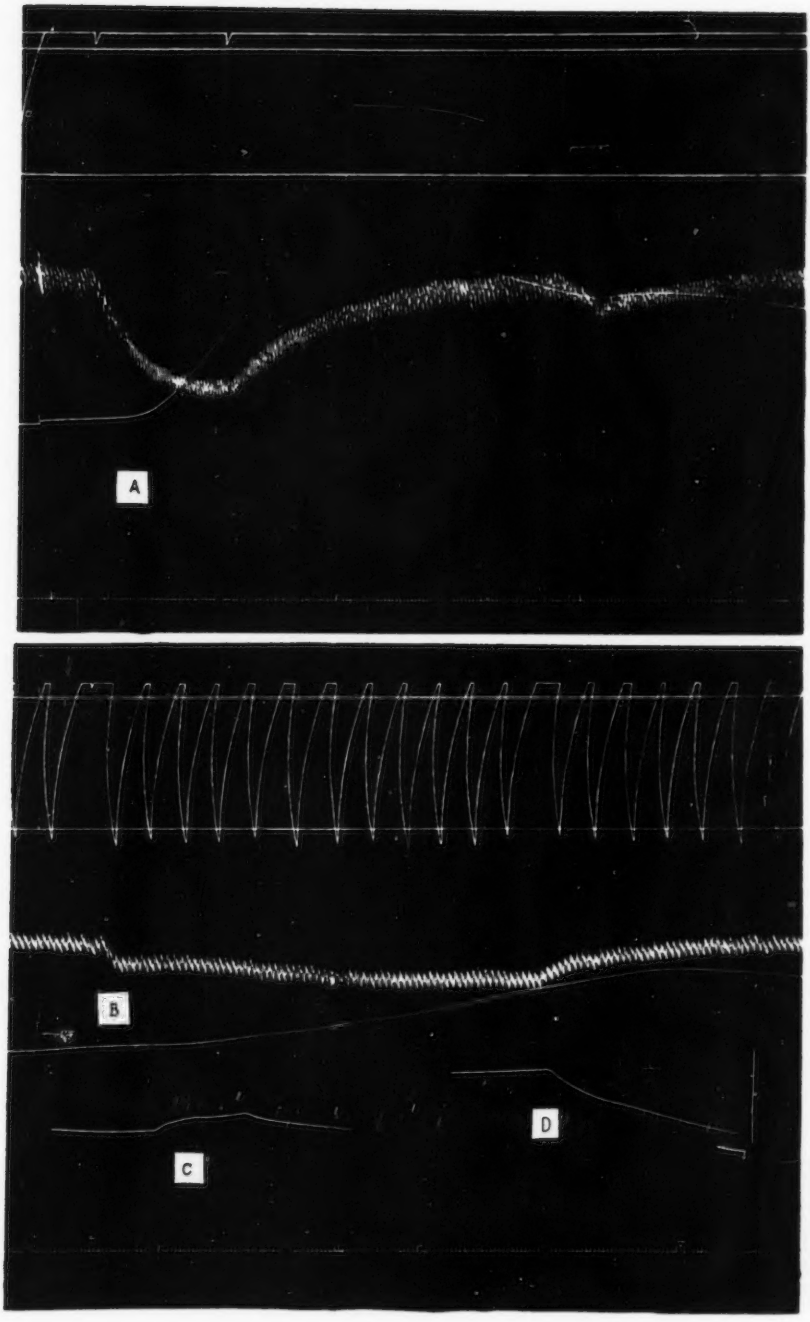

Fig. 5

through the animal's two limbs. As a matter of fact, the transverse membrane of the manometer was so easily displaced that very little differential pressure developed; instead of this, fluid passed from one side of the bridge to the other when balance was disturbed, to keep the pressure nearly equalized, and the manometer recorded more nearly the difference of *flow* through the two limbs at constant pressure.[5] Further, while the manometer was extremely sensitive to pressure changes, the volume of fluid required to move the pointer was considerable and the perfusion volume flow was relatively small in proportion. Therefore the slope of the curve written by the pointer, rather than the extent of the deflection, measured the amount of the change taking place in the animal as a result of stimulation, and the curve of the deflection integrated the difference in volume flow of the two limbs over a given time of stimulation. On cessation of stimulation the pointer, instead of returning to its base line along a curve similar to its deflection, tended only to stop deflecting, and drifted back very slowly under the force of the tension of the displaced membrane, the bridge itself being again balanced.

Total flow was measured by the amount of air sucked into the reservoir *A*, from the bellows recorder *E*, in the time required for its pointer to pass from one line on the kymograph to another after closing the side tube *F*. There was a slight lag in this method of recording, due to the fact that when the rate of flow changed, there followed a slight change of temperature in the oxygenating flask, with a consequent change of volume. The manometer recorder had no lag.

The significant experiments in this series are those in which a sympathectomized limb was compared to a completely denervated one. Bilateral sympathectomy was performed from the diaphragm to the pelvis three or four days previous to the experiment, with denervation of one limb at the time of experiment by cutting the sciatic and the femoral at the hip. All animals were autopsied to check the completeness of the operative procedure. The record of such an experiment is shown in figure 5, where the connections of the apparatus are such that a rise in the manometer curve indicates an increase of flow through the limb with dorsal root innervation intact, relative to that through the denervated limb. The changes in the limbs of the mercury manometer arms indicate further that this change was not only a relative one, but absolute. Flow increased through both limbs, the increase through the sympathectomized limb exceeding that through

[5] It is obvious that what was recorded was change neither of flow nor of pressure precisely, but a compromise, no less indicative of the events caused by nerve stimulation because of that; since any disturbance of one arm of such a bridge changes the flow through all four. But the hydrodynamics of an unbalanced bridge do not materially concern the issue, and the quantitative aspects have not been worked out further than to calibrate the apparatus with known volumes and pressures.

the denervated limb by the amount indicated on the curve. Further, since the two clamps R_1 and R_2 were not changed, the dilatation in the limbs lowered the perfusion pressure at R_3 and R_4 (fig. 4) and the increased flow thus took place in spite of a decreased head of pressure, and the greatest increase of flow, through the sympathectomized limb, was from the side of the bridge in which the pressure was lowered most. Again there is evident a delay in the onset of increased flow (fig. 5); whatever increase through both legs was secondary to the general lowering of systemic blood pressure is compensated and does not show in the record.

It still might be argued that, if collateral circulation is a factor in allowing increase of perfusion flow when the systemic pressure falls, a *difference* in the collaterals on the two sides might cause an uncompensated difference of flow not assignable to direct nervous activity. Obviously the perfusion resistances through the two legs do not have to be equal when the bridge is balanced, but only proportional to the resistances of the two pinch clamps of the bridge. In general they were not far apart, and in one successful experiment the two flows were almost exactly equal. Even if not equal, if the only cause of increased flow in the limbs were reduced systemic pressure, such passive increase of flow might be expected to be proportional to the initial flow in the two limbs, and thus the bridge would still remain balanced, but this inference is not obviously true. However, the matter could be tested. If the bridge system remained of constant resistance throughout, that is, if nerve stimulation had no effect of dilatation in the limb, a change of pressure in the systemic vessels should have exactly the same effect as a corresponding change of pressure on the perfusion bottle; either change merely corresponds to a change of net pressure applied across the whole bridge. Raising the level of the perfusion reservoir when the bridge was balanced by an amount corresponding to the blood pressure change following depressor stimulation, had no more effect than could be assigned to imperfect balance of the bridge resistance, and the effect was reversed in direction as the bridge balance was thrown off slightly in opposite directions. This we feel indicates that so far as changes passive with respect to the perfused limbs are concerned (changes in systemic pressure), the effects were compensated by the bridge system; especially since it was possible to throw the bridge definitely off balance in one direction, and still get a response to depressor stimulation in the opposite direction. What is perhaps more significant is that, upon sudden change of perfusion pressure, when the bridge was slightly off balance, the change in slope of the manometer tracing was immediate. The delay in the response of the limb to nerve stimulation, lagging behind the change in the systemic circulation, can therefore be assigned neither to delay in the action of the apparatus, nor to such a source as unequal elasticity of the two capillary beds, nor to any known irregularity of the resistance of the limbs ex-

cept that assignable to the presence in one of them of dorsal root innervation.

Usually the collateral circulation was not a predominating factor in perfusion. In one cat, however, there was an arterial anastomosis by way of a branch just below the cannula, which poured blood into the femoral artery whenever the perfusion pressure was below the systemic, and this channel was so large a part of the perfusion system as to mask any changes elsewhere.

Response to vagus stimulation. To depressor nerve stimulation in the rabbit, the response is uniformly a depression, falling at first rather rapidly, then more slowly by what may be a second process, to a level which is usually maintained as long as stimulation continues, up to at least 5 minutes. (Bayliss (1893) reports the effect to be non-fatiguable.) In some cases the pressure rose slightly after reaching a minimum. On cessation of stimulation, the pressure returns much more slowly than it falls, and may take 3 or 4 minutes to completely recover, but in our experience it never overshoots the previous normal. This offers several contrasts to the result of vagus stimulation. Even in the rabbit, with separate depressor nerve, initial depression may occur when the vagus is stimulated, but this usually is followed by a rise. In dogs and cats the rise usually predominates, but in only one case has such a rise been maintained at a constant level. Upon cessation of stimulation a further rise may take place, followed by a fall below normal, or the fall may occur at once. Vagus stimulation then, in contrast to depressor stimulation, is characterized by extreme variability. This we take to be due to the presence of both pressor and depressor afferents in the vagus, even when a separate depressor is present. For instance, one rabbit gave only pressor effects to vagus stimulation (at 5 stimuli per second) during the first part of the experiment, but later gave depressor responses to the same stimulation. The degree of anesthesia is a factor, and presumably, for lack of more specific explanation, the condition of the centers. We could not separate these effects by thresholds, and therefore infer either that the same fibers produce different effects depending on the state of the centers, or else that two groups of fibers in the vagus which have opposing results centrally are quite similar peripherally. We have not yet investigated the effects of frequency; but in the rabbit which gave only pressor effects, spraying the vagus nerve with cold Tyrode solution from an atomizer consistently gave depression, and in other cases this form of stimulus to both vagus and depressor nerves caused a fall of pressure. Since otherwise the strength of stimulus was not differential, the differential effect of cold may be assignable to a frequency effect.

More pertinent to the present issue is a result obtained in two animals where a *pressor* effect on the systemic blood pressure from stimulation of the vagus was accompanied by an *increase* of perfusion flow through the sympathectomized limb. Recognizing that the vagus has both pressor and de-

pressor pathways, and on the assumption that the usual complex response to electrical stimulation involves the addition of two antagonistic influences, the above result suggests further that the antagonism may be peripheral as well as central. Stimulation of the pure depressor obviously decreases sympathetic tone, as well as activating dilators, and stimulating a pure pressor pathway might be expected to do just the reverse; the possibility arises that stimulating both pressor and depressor afferents together under certain conditions may activate both sympathetic constrictors and dorsal root dilators at the same time, the actual dilatation of vessels occurring only in the sympathectomized limb. This would not be the normal occurrence perhaps in the body, where selection of pressor or depressor afferents would be determined by afferent end organs.

In a rabbit where vagus stimulation gives a predominant pressor effect, stimulation of both vagus and depressor coincidentally merely lowers the vagus curve, without materially altering its form. In view of the one experiment where we obtained from the vagus a pure pressor effect, which was maintained during stimulation at constant level, one might infer that pressor effects as well as depressor effects were non-fatiguable, and that the customary failure to obtain sustained and consistent effects from either vagus or other peripheral nerve stimulation might be due to the fact that electrical stimuli usually activate both pressor and depressor afferents. These effects are being further investigated.

Discussion. For purposes of discussion, we wish to present our interpretation of these experiments under the following theses. First, there is a specific group of fibers in the dorsal roots with cells of origin in the dorsal root ganglion for which we can find no afferent function, under conditions where all other fiber groups show specific effects of stimulation. Second, these are small fibers, as indicated by the order in which they are blocked by narcotics and by pressure; they are inferred to be non-myelinated fibers for reasons stated; they are certainly not responsible for mediation of pain. Third, a reflex vasodilatation taking place in the sympathectomized limb is assigned to these fibers as motor fibers, because Hinsey and Gasser showed that these were the only fibers in the dorsal root whose direct stimulation causes vasodilatation, and because similar and in fact anatomically homologous fibers in the vagus have a motor function (Heinbecker and O'Leary, in press).

Regarding the first point, the possibility of course exists that some afferent function not yet recognized may be served by these fibers, but the burden of proof would seem to rest upon those so obsessed with the Bell-Magendie "law" that they have forgotten that even this law was only established by experiment; in fact by stimulation experiments quite analogous to ours. A priori there seems to be no reason for considering these fibers sensory, except by analogy with other known sensory fibers, which take the

the same course. Lacking any obvious sensory response, one might look for an effect of stimulation of these fibers upon the effects of stimulation of other sensory fibers; that is, these fibers, without causing any specific sensation themselves, might condition the response to other afferent excitation. The most obvious sensation to study in this respect would be pain, since these fibers have a similar course to that of pain fibers, but no practical means of doing this has occurred to us yet. The fact that the fifth nerve is capable of mediating pain over a path (spinal Vth) with very few non-myelinated fibers (Windle, 1926) raises doubts as to the probable value of such a study.

Degeneration of the dorsal roots results in degeneration of these non-myelinated fibers in the medial division of Lissauer's tract (Ranson, 1913), and they appear to terminate in the substantia gelatinosa. Fibers also are known to pass between this Lissauer tract region and the lateral areas of the cord. The lateral areas at cord levels giving rise to autonomic fibers, contain their cell bodies. The direction in which impulses pass over these fibers has not been determined.

The second conclusion involves a difference of interpretation from that offered by Ranson (1931) that the group of *small* fibers, both myelinated and non-myelinated, can be treated as a functional unit. Anatomically they have similar pathways, so far as they have been traced; physiologically two quite different groups of fibers can be separated, with different properties, and with no transitional stages between them, both certainly assignable to a range of diameters below 5 u. Functionally, stimulation of one group results in pain and reflex effects, stimulation of the other does not. This obviates the difficulty Ranson met with in the spinal Vth tract, where a paucity of non-myelinated fibers compromised the generalization that non-myelinated fibers were responsible for pain. With our finding that pain elsewhere is mediated by fibers of the sensory B group, not by C fibers, Windle's demonstration that pain must be mediated by myelinated fibers offers support to our assignment of the B_1 or sensory B group to small myelinated axons.

In regard to vasodilatation, the greatest difficulty appears to us to arise from the *number* of non-myelinated fibers present. Ranson has shown that the number is greater than that of all other fibers in the saphenous nerve, and estimations based on C potentials of other nerves as compared with the saphenous point also to a large number of C fibers in the latter. But while a comparison of the number from the dorsal roots with that from the sympathetic rami, either by actual counts of axons or by areas of action potentials, indicates a numerical ratio of 5 or 10 to 1 (Heinbecker, O'Leary and Bishop, 1933), our experiments on vasomotor function indicate that the sympathetics have a much greater influence than do the dorsal roots. If all the fibers under discussion are vasodilator fibers, the effect per fiber is relatively insignificant.

The possibility that dorsal root C fibers may not *all* be vasodilators, as the sympathetic fibers are not all vasoconstrictors, would be more cogent if some other effect of antidromic dorsal root stimulation were known: we have observed none, but propose to look further in this direction. On the other hand, this effect from the dorsal roots has been assigned to capillaries rather than to arterioles (Langley, 1923) and if the number of sympathetic fibers required to constrict arterioles were compared with the number which might be required to dilate the vastly more numerous capillaries, the discrepancy might not be so great. This explanation is complicated, however, by the proposal of Langley (discussed by Hinsey and Gasser) that whatever vasodilatation is produced by dorsal root stimulation shows all the signs of being an indirect or hormonal one, and not a direct effect of innervation of vessels. No one can predict how many nerve fibers should be required to cause the production of a given humoral substance, and even less, how much effect this substance should have. On the other hand, we doubt whether either plethysmographic or perfusion experiments as we have done them, are adequate to estimate the importance of the dorsal root dilator mechanism in the normal animal. As is well known, blood volume flow is largely controlled by arterioles rather than by capillaries. In the intact animal the presence of the sympathetic innervation to arterioles may mask the effect of the dorsal roots on capillaries. In the sympathectomized animal the circulation adjusts itself in part after an initial dilatation, although the temperature of the limb remains high. It may be that after this adjustment, which is a pressor effect, a depressor influence is no longer exerted with its normal effectiveness. It is also possible that a *local* reflex activation of dorsal root dilators would be more effective than a general activation via the depressor nerve; the juxtaposition of the dorsal root non-myelinated fibers and pain fibers in the cord might suggest a local association. We can only recognize that there seem to be a great many fibers involved in producing a relatively slight effect as measured by volume flow; and we hope to be able to study the vasomotor reactions of sympathectomized animals to normal stimuli, rather than to direct nerve stimulation, to determine what advantage the animal might gain by such a mechanism, that would be appropriate to the odd way in which he appears to bring it about.

SUMMARY

Direct electrical stimulation of the slowest conducting group of fibers in the saphenous nerve of lightly anesthetized or decerebrate dogs and rabbits fails to cause any detectable reflex response. This obtains when, by differential pressure block, the activity of all fibers except these is suppressed.

Evidence is reviewed indicating that these fibers, the so-called "C" fibers,

are the non-myelinated ones, including both sympathetic and dorsal root components.

Stimulation of a small fraction of the fibers of the next faster conducting group, the rest being blocked, gives respiratory, motor and vasomotor reflexes, and in non-anesthetized animals, obvious pain, under conditions of differential block similar to the above, and the extent of the response increases with the number of fibers activated in this group. The fibers are small myelinated ones, and include those mediating temperature sensations.

Plethysmographic volume of the sympathectomized limb increases relatively to the volume of the denervated limb upon stimulation of the depressor nerve in lightly anesthetized rabbits, although absolutely the volumes of both may decrease due to the marked lowering of systemic blood pressure.

During differential perfusion with Tyrode's solution of the two hind limbs of rabbits, one sympathectomized and one denervated, stimulation of the depresssor nerve results in a delayed increase of flow through the sympathectomized limb, greater than the more immediate (passive) increase through both limbs assignable to decreased systemic pressure. This delayed increase resembles that obtained by so-called "antidromic" stimulation of the dorsal roots.

It is concluded that the non-myelinated fibers of the dorsal roots of the saphenous and similar peripheral nerves include motor vasodilator fibers, with cells of origin in the dorsal root ganglia, accessible to reflex activation via synapses within the cord, and that none of such fibers mediate any of the commonly tested afferent functions; and in particular, that all types of pain may be accounted for without involving such fibers.

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RESPIRATORY METABOLISM OF ACTIVE AND INACTIVE RATS

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In the spring of 1928 one of us (Rundquist) began the isolation by selective breeding of two strains of rats—one strain breeding true for relative activity, the other for relative inactivity. The apparatus used to obtain a measure of this spontaneous[1] activity was a revolving drum of the squirrel-cage type, first devised by Stewart (1898) and shown to give a reliable measure by Shirley (1928). The record is obtained from counters attached to the cages, recording each complete revolution in either direction. A 21 day run utilizing the last 15 days' record was found to be sufficiently reliable for the purposes of this experiment. From table 1, which gives the activity averages and standard deviations of the F_{15} animals used in the present experiment, it is clear that the two strains are well isolated. Such homogeneous groups at the extremes of the activity distribution offer ideal material for further experimentation involving the physiological bases of activity; and in the following experiment we attempted to determine to what extent basal metabolism is related to this activity. While, to the authors' knowledge, there is no direct work on the relation between the two in the rat, indirect evidence has led workers in the field to conclude that basal metabolism and activity are independent in their variation (Lee and Van Buskirk, 1928).

TABLE 1

Activity means (in hundred revolutions for 15 day period) and standard deviations (σ) of active and inactive animals

	ACTIVE		INACTIVE		DIFF.	$\frac{\text{DIFF.}}{\sigma \text{ DIFF.}}$
	Mean	σ	Mean	σ		
Males	1,250.00 (29)	630.23	34.00 (16)	48.75	1,216.00	10.34
Females	2,013.05 (20)	668.08	197.91 (22)	198.98	1,815.14	11.68

The numbers in parentheses indicate the number of cases.

[1] By spontaneous is simply meant that the stimulus to activity is internal rather than external.

APPARATUS AND METHOD. Respiratory metabolism was determined by the technique described by Ebeling and Corey (1930), modified to suit our purposes, the changes consisting principally of enlargements of the parts and increased reagent volumes. The glass chamber employed was of 5000 cc. volume, 400 cc. of water being added at the beginning of each determination. A sponge rubber seal was employed between the rim of the jar and the brass cover as an additional precaution against gas leakage. A preliminary equilibratory period of 10 minutes preceded the 20 minute test period. Anesthetics were found to be unnecessary, the cage being so constructed as to fit any size rat introduced into it. This was accomplished by a stiff flap of aluminum alloy which kept the rat in place and allowed a minimum of movement, the animal usually sleeping during the thirty minute test period. One hundred or 150 cc. of base were added at the beginning depending upon the weight of the animal, and additional base, to compensate for O_2 usage, was added automatically by allowing the burette to drop a constant number of drops per minute.

Basal metabolic rates were determined at least two days after the animal had been removed from the activity cage in which he had been during the preceding twenty-one days. A fasting period of at least 12 hours preceded the determination.

RESULTS. The significant figures for the metabolic rate of the active and inactive groups are given in table 2. The most significant point to be noted from this table is the difference in basal metabolic rate between the active and inactive animals; in both males and females the basal rate of the active is about 2 Calories per gram per hour higher than that of the inactive. These differences are of statistical significance. A comparison (justifiable since the same animals were involved in each computation) of the D/σ diff. ratios for activity (10.34 for males and 11.68 for females) and basal metabolism (4.06 for males and 4.85 for females) suggests that other factors besides basal metabolism are involved in activity. This is also indicated by the absence of a marked sex difference in basal metabolism (table 2), whereas the sex difference in activity is pronounced (table 1).

TABLE 2

Basal metabolic rates (Cal./gm./hr.) of active and inactive groups two days after removal from activity cages

	ACTIVE		INACTIVE		DIFF.	DIFF./σ DIFF.
	Mean	σ	Mean	σ		
Males	8.25	1.05	6.28	1.79	1.97	4.06
Females	8.70	1.51	6.45	1.47	2.25	4.85

Number of cases as in table 1. Calories computed from table given in MacLeod: *Physiology and Biochemistry in Modern Medicine*, C. V. Mosby Co., St. Louis, 1922, p. 599.

In fact the sex difference in basal metabolism, while favoring the most active sex, the female, is not of sufficient magnitude to be considered a true difference, unless repeatedly confirmed; while the sex difference in activity at the ages involved in this experiment is well established (Rundquist).

In order to determine whether the relation between basal metabolism and activity could be detected within each strain (obviously the two strains represent two distinct samples and hence cannot be combined for this computation) the following rank-order correlations between the two were computed: active males, −0.21; active females, +0.29; inactive males, −0.09; inactive females, +0.13. These correlations are based on so few cases that none of them can be considered to deviate significantly from zero. Either the animals of each strain are so much alike in basal rate, in activity, or in both that the variability in one or both is due to chance environmental factors, or it takes a larger variation than found within these strains in one to affect the other. There is, of course, the possibility that these correlations are due to chance and that a repetition of the experiment might show a moderate correlation within the strains. The fact that all four are low does not support this supposition.

TABLE 3

Weights, ages, and respiratory quotient of active and inactive animals

	WEIGHT		AGE		R.Q.	
	Mean	σ	Mean	σ	Mean	σ
Males						
	gm.	*gm.*	*days*	*days*		
Active	174.27	25.09	89.21	5.40	0.81	0.057
Inactive	177.70	20.38	84.94	2.81	0.81	0.094
Females						
Active	117.00	12.89	83.00	2.18	0.87	0.013
Inactive	127.23	10.88	85.32	2.18	0.97	0.016

Number of cases as in table 1.

In table 3 are given other data for these same animals in order to assist in understanding the above difference in basal metabolic rates between the active and inactive groups. Age is not a contributing factor, the age differences between the groups being very slight. For the males there is no difference in respiratory quotient between active and inactive groups, but the respiratory quotient of the inactive females is higher than that of the active females by 0.10. Later in this paper data will be presented to show that this difference is probably of little significance. The weights of the active and inactive males are very similar, while inactive females are,

on the average, about ten grams heavier than the active. It is not probable that the small differences between the female groups are of any great significance. The possibility that the higher basal rates of the active animals were the result of their previous greater exercise is rendered unlikely by the work of Steinhaus (1927–28; 1933) which indicates that, following exercise, the basal metabolic rate is either lowered or unchanged. It seems, therefore, that the two strains differ in basal metabolism as well as in capacity for spontaneous activity and that basal metabolism is one of the physiological factors underlying this activity. These results and inferences are in accord with the observations of Rockwell and Rundquist (in preparation) that while thyro-parathyroidectomy markedly reduces activity, parathyroidectomy has no appreciable effect.

Although it had been originally planned to obtain basal metabolic rates for both active and inactive groups before, as well as after, they had been placed in the activity cages, we were only able to make determinations on a few of the inactive group at that time. Our main comparison, which we have discussed above, is between determinations taken following an initial 21 day run in the cages. Immediately following the animals were replaced in the activity cages for an additional 21 days. Following this, we were able to make a few further basal metabolic rate determinations, table 4 containing the data for the animals on which we made more than one determination. While there are too few cases in this group to warrant a statistical analysis, it is worthy of notice that the basal metabolic rates decrease markedly in all groups despite the small age difference involved.

TABLE 4

Average age, weight, basal metabolic rate, and respiratory quotient of the same animals at different ages

AGE		WEIGHT		B.M.R.		R.Q.	
Active	Inactive	Active	Inactive	Active	Inactive	Active	Inactive
Males							
		(9)	(8)	(9)	(8)	(9)	(8)
		gm.	*gm.*				
—	59	—	119	—	6.79	—	0.78
85	84	158.5	186	8.79	5.28	0.82	0.79
113	113	207.3	221	5.55	3.72	1.02	0.88
Females							
		(5)	(9)	(5)	(9)	(5)	(9)
—	58	—	90	—	6.71	—	0.88
85	84	115.2	127	8.54	5.26	0.98	1.02
112	112	138.5	151	6.87	5.10	0.90	0.86

The numbers in parentheses indicate the number of cases.

The active groups are at all times higher than the inactive. Whether the decrease in basal metabolic rate is solely a function of age cannot be determined from the data at hand.

In order to determine to what extent daily variations in basal metabolic rate might have influenced our results and to get some check on the reliability of our technique, daily observations were made for two active (A) and two inactive (I) males for a period of two weeks. No access to food was allowed for a twelve hour period preceding the determination. Table 5 contains these data. It is clear that, although the respiratory quotient is highly inconstant, varying between 0.70 and 1.20 in the same animal, the basal metabolic rates are fairly constant over this period. The basal rates of the active animals are definitely higher than those of the inactive, approximating the averages obtained for the groups. The basal metabolic rate of none of the rats involved varies sufficiently to mistake the strain to which it belongs. The variation in the respiratory quotient is somewhat greater than that observed for men by Paulton, Spurrel, and Warner (1929). They found the quotient to vary between 0.84 and 0.92 in the same individual over a thirteen day period.

TABLE 5

Respiratory quotients and basal metabolic rates (Cal./gm./hr.) of two active (A) and two inactive (I) male rats, observed daily over a period of two weeks

No access to food was allowed for a twelve hour period preceding the morning of each determination.

DAY	RAT 57 (I)		RAT 98 (I)		RAT 23 (A)		RAT 40 (A)	
	R.Q.	B.M.R.	R.Q.	B.M.R.	R.Q.	B.M.R.	R.Q.	B.M.R.
1	0.93	6.01	0.92	6.30	0.78	8.75	0.88	7.89
2	0.87	6.00	0.82	5.82	0.77	8.00	0.90	9.20
3	0.90	5.95	0.80	5.93	1.11	7.75	0.70	8.88
4	1.00	6.34	0.77	5.98	1.20	7.89	0.73	8.28
5	0.88	6.30	0.70	6.12	0.88	8.00	0.90	7.90
6	0.83	5.99	0.86	6.13	0.83	8.00	0.88	8.00
7	0.85	6.12	0.84	5.54	0.88	8.11	0.83	9.35
8	0.94	6.32	0.98	5.87	0.82	8.06	0.82	9.69
9	0.70	6.44	1.20	5.88	0.87	8.88	0.92	8.38
10	0.83	6.16	0.75	5.96	0.80	7.65	0.90	8.88
11	0.92	6.10	0.83	6.01	0.83	8.77	0.95	9.34
12	0.87	6.24	0.84	6.22	0.86	8.68	0.96	9.77
13	0.90	6.00	0.80	5.88	0.86	8.01	0.88	9.01
14	0.83	5.97	0.90	5.87	0.90	8.00	0.82	9.20
Activity record in 100's for a 15 day period		0		0		2837		3110

SUMMARY

Basal metabolic rates were determined on relatively homogeneous groups of active and inactive rats from the fifteenth generation of strains selected on the basis of spontaneous activity. The basal metabolic rates of the active animals were higher, while there were no significant differences in age, weight, or respiratory quotient. Successive determinations on a few animals of each group revealed that the basal metabolism drops markedly in a period of about three weeks. At all times, however, the rates of the active animals were higher. Daily determinations for two active and two inactive male rats over a two weeks' period indicated that the basal metabolic rate is a fairly constant figure while the respiratory quotient is highly variable. While there is a marked sex difference in activity, no such difference was found in basal metabolic rates.

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THE ENERGY METABOLISM OF PHOSPHORUS-DEFICIENT DAIRY CATTLE[1]

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One of the interesting problems in connection with phosphorus deficiency disease in cattle is that of lowered feed utilization. This has been demonstrated experimentally by Theiler and co-workers (1924) in South Africa and Eckles and associates (1926) in Minnesota.

It has already been shown by the authors (1933) that the inefficient utilization of feed is not due to failure on the part of the affected animal to properly digest its feed or inability to completely metabolize the products of digestion. It would appear, therefore, that this failure to utilize the feed in a normal manner in the phosphorus-deficient condition must be due to an increase in the rate of metabolism. Theiler (1924) in discussing this problem suggests that affected animals are "simply combusting the extra ration for no useful purpose."

While considerable investigation has been made of the energy metabolism in cattle on different planes of nutrition, no record is available of any such studies with cattle in advanced stages of aphosphorosis. In order to secure data on this important question the present investigation was undertaken.

EXPERIMENTAL. Recently Brody and associates (1930) have adapted the Benedict-Roth recording metabolism apparatus for use with farm animals. The respiratory system of the animal is connected with an oxygen spirometer by means of a rubber sleeve pulled over the animal's muzzle to serve as a mask.

Since this type of apparatus has proved satisfactory for studying the energy metabolism of dairy cattle, it was decided to make use of it in the present investigation. Through the courtesy of the Department of Dairy Husbandry, University of Missouri, a portable respiration apparatus was secured.

Two lactating dairy cows, E-13, fresh December 15, and E-16, fresh

[1] This paper represents a portion of the thesis presented by W. H. Riddell in partial fulfillment of the requirements for the Ph.D. degree, University of Minnesota, 1932. Contribution no. 89, Department of Dairy Husbandry and no. 177, Department of Chemistry.

January 15, were used in this study. Both were senior two-year olds and were producing approximately 40 pounds of milk each per day at the outset of the experiment. These cows were placed on the phosphorus-deficient ration in March, 1931, and were in advanced stages of aphosphorosis when the first measurements were made the following May.

It was planned to have the experimental animals serve as their own controls in that following the collection of satisfactory oxygen-consumption records in the phosphorus-deficient condition, the same animals were to be

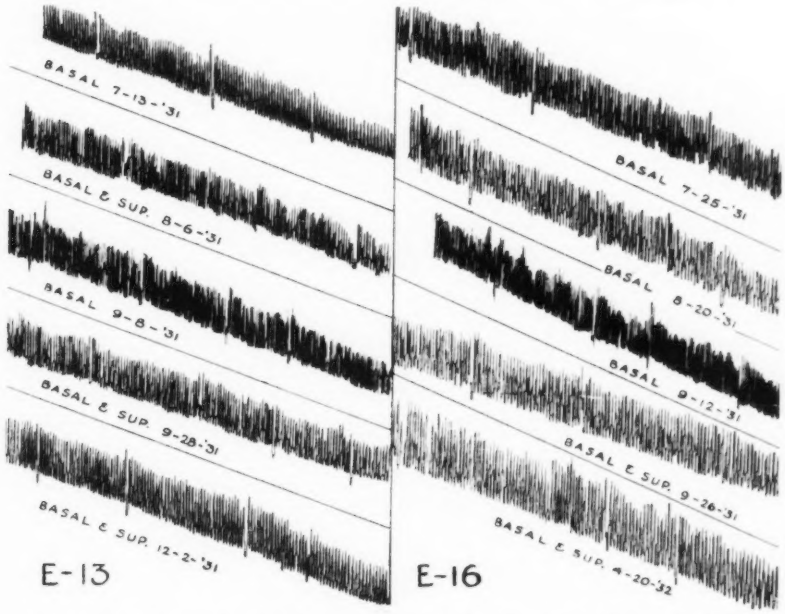

Fig. 1. Typical records of oxygen consumption for experimental cows during the periods of low phosphorus (basal) and supplemented feeding.

fed the phosphorus supplement (100 gm. of monosodium phosphate daily) and similar records obtained in the supplemented condition.

For the most part the plane of feeding of the experimental animals was approximately 110 per cent of the estimated digestible nutrients required as prescribed by the Morrison feeding standard (1928). The low phosphorus ration, which contained approximately 0.18 per cent phosphorus, was comprised of the following feeds: alfalfa hay, prairie hay, beet pulp, cane molasses, blood flour, yellow corn, and oats. Water and salt were always available. The cows were maintained in dry lot throughout the the experiment, being turned into the barn only for feeding and milking.

In making metabolism measurements it is essential that the subject be as completely relaxed and quiet as possible. Considerable difficulty was encountered in training the animals used in the present work to lie down and remain quiet while the measurements were being made. After approximately three months of careful training the animals became accustomed to the procedure and would perform in a manner permitting the securing of satisfactory records.

Typical records of oxygen consumption for each cow during the periods of low phosphorus and supplemented feeding are shown in figure 1.

A summary of the results calculated from the oxygen consumption records is presented in table 1.

Discussion of results. It is apparent from a study of the data in table 1 that there is a general tendency for higher oxygen consumption, indicating a higher rate of metabolism, with the animals in the phosphorus-deficient condition. With the addition of phosphorus supplement to the ration, their metabolism tends to decline.

It should be emphasized that while the experimental cows were fed about the 110 per cent level of nutrient intake as prescribed by the Morrison feeding standard (1928) loss of appetite was a factor of considerable importance early in the period of low phosphorus feeding. Before a sufficient number of satisfactory records could be secured on these animals in the phosphorus-deficient condition, it was found necessary to bring about partial recovery by the addition of the supplement (100 gm. monosodium phosphate daily). This was done because of the general weakened condition of the cows and also to stimulate their appetites. A period of phosphorus feeding extending from July 21 to August 20, 1931, is recorded for E-13, while for E-16 it was relatively short (Aug. 26 to Sept. 4).

It is significant that a decrease in the rate of metabolism is indicated for E-13, the only animal on which measurements were made, during this period of supplemental feeding. With the withdrawal of the supplement from the ration there was a significant rise recorded in the rate of metabolism. On September 19 both cows were placed on the supplemented ration and continued in this manner to the end of the experiment. This resulted in a substantial decline in the oxygen consumption of both cows, indicating that the addition of phosphorus was in some way responsible for a lowering of the general metabolism of the individual.

The metabolic stimulus of food is well recognized. It is reasonable, therefore, to expect that the metabolism of these cows probably would have been higher than the results obtained in the early months of the experiment, had they been consuming the full amount of the ration offered.

Throughout the period in which these measurements were made there was a considerable decrease in the lactation level of the experimental cows. Since Brody and associates (1932) have stated that lactating dairy cows

TABLE 1

Energy-metabolism data

DATE	RATION	WEIGHT	AREA* (A)	HEAT PRODUCTION, CAL. FOR 24 HOURS			BARN TEMPERATURE
				Total	Per sq. m.	Per kgm.	
E-13							
		kgm.	*sq. m.*				*°C.*
5-29-'31	Basal—low-P	349	3.98	11,346	2,851	32.6	20.0
7-13-'31	Basal—low-P	340	3.92	10,535	2,688	30.9	23.0
7-21-'31	Basal—low-P	353	4.01	11,140	2,778	31.5	23.5
7-29-'31	Basal + sup.	360	4.05	10,351	2,556	28.8	27.0
8-1-'31	Basal + sup.	361	4.05	10,696	2,641	29.6	25.0
8-6-'31	Basal + sup.	359	4.05	10,284	2,539	28.6	25.0
9-8-'31	Basal—low-P	384	4.20	11,287	2,687	29.4	27.0
9-10-'31	Basal—low-P	386	4.21	13,328	3,166	34.5	25.5
9-12-'31	Basal—low-P	355	4.02	13,802	3,433	38.8	25.0
9-28-'31	Basal + sup.	391	4.24	10,541	2,486	27.0	17.0
11-30-'31	Basal + sup.	432	4.48	12,151	2,712	28.1	10.0
12-2-'31	Basal + sup.	426	4.45	12,909	2,901	30.3	8.0
3-1-'32	Basal + sup.	470	4.70	12,742	2,711	27.1	16.0
4-19-'32	Basal + sup.	509	4.92	12,572	2,555	24.7	15.0
Average of six low-P determinations				11,906	2,934	32.9	—
Average of eight supplemented determinations				11,531	2,637	28.0	—
E-16							
5-29-'31	Basal—low-P	383	4.19	12,586	3,004	32.9	21.0
7-18-'31	Basal—low-P	350	3.98	11,544	2,901	32.9	25.0
7-25-'31	Basal—low-P	343	3.94	11,351	2,881	33.1	24.5
8-20-'31	Basal—low-P	330	3.85	12,994	3,375	38.6	21.0
9-9-'31	Basal—low-P	347	3.97	11,710	2,950	33.7	26.0
9-12-'31	Basal—low-P	337	3.90	14,416	3,696	42.8	24.5
9-26-'31	Basal + sup.	340	3.92	11,182	2,853	32.9	14.5
11-22-'31	Basal + sup.	381	4.16	12,257	2,932	32.2	13.0
2-27-'32	Basal + sup.	420	4.42	12,538	2,876	30.0	14.0
4-20-'32	Basal + sup.	470	4.70	12,472	2,654	26.5	15.0
Average of six low-P determinations				12,434	3,135	35.7	—
Average of four supplemented determinations				12,156	2,829	30.5	—
E-13 + E-16							
Average of 12 low-P determinations				12,169	3,304	34.3	—
Average of 12 supplemented determinations				11,739	2,701	28.8	—

* The surface area was computed according to the formula $SA = 0.15\ W^{.56}$ (Mo. Research Bull. 89), in which SA is the surface area in square meters and W the weight in kilograms.

show a metabolic level 30 to 60 per cent above the general level of metabolism, it might be inferred that part of the decrease in oxygen consumption could be accounted for by the lowered intensity of lactation. Brody, however, suggests that "the causative factor for the high metabolism during lactation may not be the process of lactation as such, but chiefly the heat increment of the extra food consumption during lactation."

In the present study the feed consumption was not reduced as the level of milk production decreased, but was increased soemwhat on each occasion that the phosphorus supplement was added to the ration, because of the improved appetites of the cows. On this basis, the change in lactation level would not account for the decrease in metabolism observed in both cows toward the end of the experiment.

Average values for the metabolism of each cow in terms of total heat production for 24 hours and in relation to body weight and surface area have been given in table 1. It will be noted that the energy metabolism for E-16 was on a higher level than for E-13 in both the low phosphorus and supplemented conditions. This is probably explained in part by the better appetite of the former, resulting in a higher food consumption, especially in the periods of low phosphorus feeding.

The general summary of the energy metabolism values for both cows during the period of low phosphorus feeding gave an average figure for the two animals of 3,034 Calories per square meter of body surface and 34.3 Calories per kilogram per 24 hours as contrasted with similar determinations on these cows in the supplemented condition of 2,706 Calories per square meter and 28.8 Calories per kilogram.

The higher metabolism of the phosphorus deficient cows would help to explain the lowered efficiency in feed utilization of animals afflicted with this disorder. It is recognized, however, that the relatively few determinations presented in this paper are not sufficient to establish the magnitude of the effect of phosphorus deficiency on metabolism. Additional data, both as to number of animals and measurements involved, will have to be secured before it is known whether increased metabolism can account for the entire difference in the utilization of feed by the normal and the phosphorus-deficient animal.

SUMMARY

The results of an investigation, studying the problem of lowered feed utilization in phosphorus-deficient cattle, are reported.

Oxygen consumption measurements made by means of a portable metabolism apparatus indicated a higher energy metabolism for animals in the phosphorus-deficient condition.

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STUDIES ON THE ORIGIN OF THE AUTOMATICITY OF THE INTESTINE: THE ACTION OF CERTAIN DRUGS ON ISOLATED INTESTINAL TRANSPLANTS

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A series of studies dealing with muscular activity and electropotential variations of the small intestine has been carried out on dogs. Segments of small bowel at various levels were isolated and transplanted to the surface of the abdomen by a technique previously described (1). Bipolar electrographic records were obtained by applying electrodes attached to a string galvanometer to the serosal surface of the intestinal segments. A simultaneous myographic record obtained by an air-filled rubber balloon in the lumen of the segment was adjusted to record on the same film with the electrogram. Waves of altered electrical potential of a fairly characteristic form occurred at a rate constant for a given segment under most physiologic conditions but gradually diminishing from the pylorus to the ileo-cecal region. A definite time relationship exists between the mechanical myogram and the electrogram when both are present but the waves of altered potential persist when no record of muscular activity can be obtained. Previous studies have shown that these potential changes probably arise from some cause within the segment itself because when all original connections with the central nervous system have been severed they persist in practically an unaltered form. The two most likely sources of this phenomenon are the intestinal musculature and the intrinsic nervous tissue which consists of the plexuses of Auerbach and Meissner. In an effort to obtain further information regarding the nature and origin of the electrogram a series of drugs was administered and the effects noted.

The intravenous administration of a solution containing 0.005 mgm. of epinephrine per kilogram of body weight was followed by a complete cessation of the myogram within ten seconds (fig. 1-A). There was a marked relaxation of intestinal tonus and no visible muscular activity. The electrogram however persisted at approximately the same rate and with little variation in form except in the prolonged phase of the wave which corresponds in time to visible muscular activity. With the cessation of muscular activity this phase did not present the fine oscillations

which occur when there are visible contractions. The myogram rapidly returned and was normal in about five minutes. Larger amounts of the drug produced similar results but also diminished the amplitude of the electrogram especially one to two minutes after administration (fig. 1-B). The rate was increased slightly immediately after the drug was given but soon slowed to a little below normal. Alvarez (2) and Berkson (3) have noted quite similar results of this drug on the intestine of rabbits.

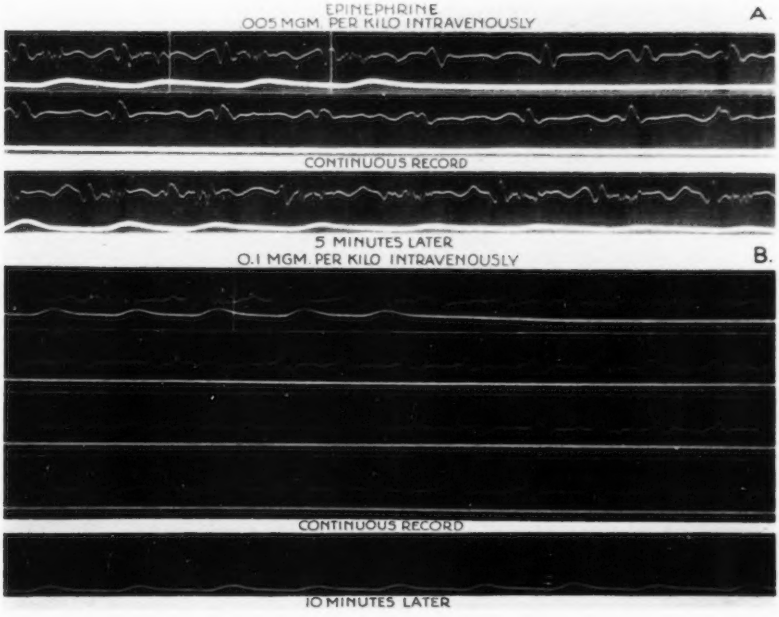

Fig. 1

Figs. 1–5. Upper record is electrogram. Lower record is myogram. Time intervals 1 second. Signals indicate time of administration of drugs.

Eserine (0.1 mgm. per kgm.) was followed within thirty seconds by an increase in degree and duration of the contraction phase of the myogram but by no change in the electrogram except for an increase in number and amplitude of the fine oscillations in the part of the wave which corresponds in time to the muscular contraction (fig. 2). When an equal amount of atropine was given following eserine the strength of contractions as recorded on the myogram rapidly diminished and within thirty seconds no activity was visible. The tonus of the segment also diminished. The contractions returned in less than two minutes but were not as strong as

before the administration of atropine. The electrogram was unchanged in either rate or amplitude, the only change in form being the absence of the oscillations which accompany muscular contractions. When atropine was administered with no drug preceding it there was a cessation of the

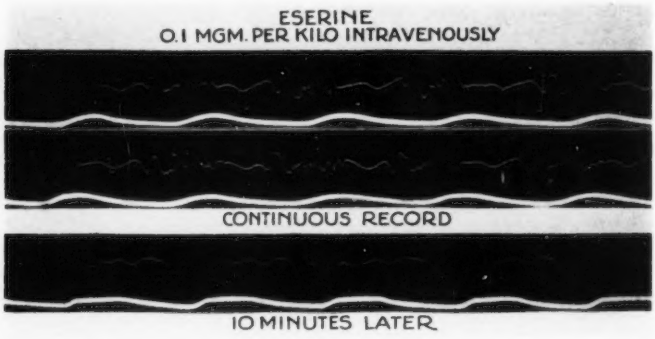

Fig. 2

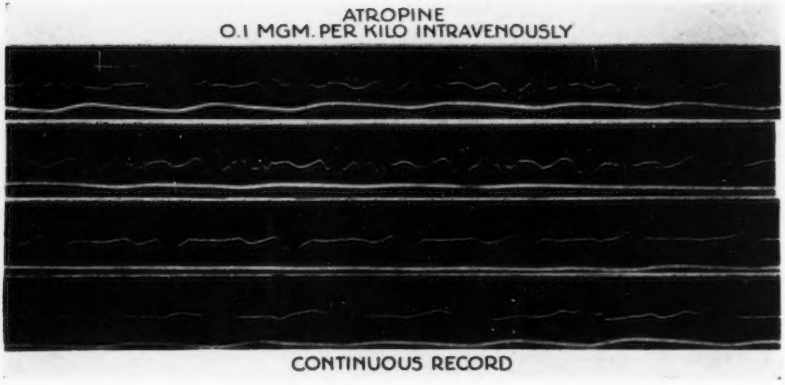

Fig. 3

myogram for about one minute after which it gradually returned to normal. The electrogram was practically unaltered (fig. 3).

Ergotamine tartrate (0.1 mgm. per kgm.) was followed by a gradual increase in the tonus of the intestinal segment and in the strength of its contractions (fig. 4). The maximum effect was reached within two minutes and was greater than has been noted under any conditions studied. The electrogram showed marked oscillations during the phase simultaneous

with contraction of the bowel. Vomiting occurred at this time. The effect of the drug on the myogram was still quite evident although somewhat diminished thirty minutes after its injection. There was a very slight slowing of the rate at this time. This drug was used after a 10 per cent solution of cocaine had been applied to the segment and at this time was also followed by increased activity.

Cotton was saturated with solutions of cocaine and applied within the lumen of the bowel and over its outer surface. The strength of the solution was gradually increased from 1:1,000 to 10 per cent. The electrogram and myogram continued and no constant change was noted which

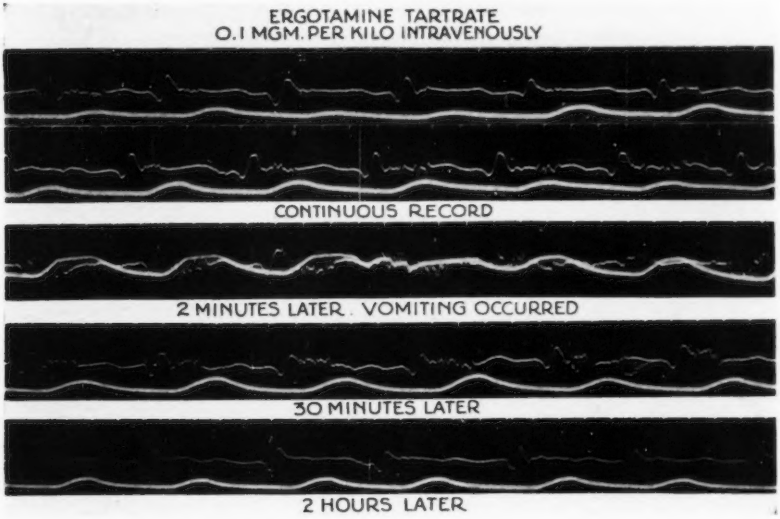

Fig. 4

could be definitely attributed to the drug. A 1 per cent solution of nicotine was kept in constant contact with the lumen of the isolated intestinal segment by means of saturated cotton and electrograms were made. No changes were noted and the solution was then applied to the outside of the bowel also. Following this application vomiting occurred. The electrogram was practically unaltered. The nicotine solution was then injected into the wall of the bowel segment by means of a hypodermic needle. This was followed by profuse vomiting, rapid respiration and tachycardia. However, the electrogram continued to show waves of altered potential.

A series of animals, under sodium pentobarbital anesthesia and with respiration maintained artificially, were given nicotine intravenously in

dosages up to 10 mgm. per kilo of body weight repeatedly. This rapidly was followed by partial or complete cessation of both the electrogram and myogram. When activity was recorded a relationship between the electrogram and myogram persisted but the rate and amplitude of both were markedly diminished. When both records showed complete cessation of activity the inactivity persisted for one-half to several hours before gradually returning. The rate remained slow and the amplitude of both remained small. Slight potential variations occurred at times with no visible associated muscular activity but no contractions were noted which did not have an associated potential change. However this was often very slight. Barium chloride administered intravenously following nico-

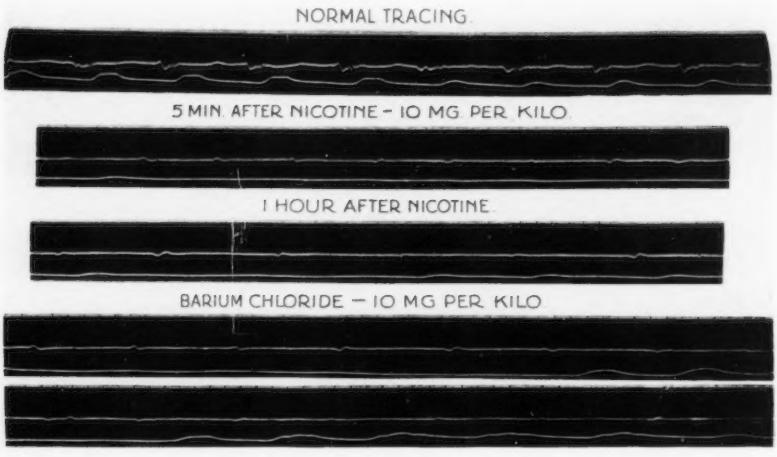

Fig. 5

tine greatly increased the strength of muscular contractions but the potential changes remained very slight (fig. 5).

Two preparations of the pituitary gland were used, pituitrin (5 units) and pitresin 1 cc. intravenously. Both produced a similar effect. The muscular contractions as shown by the myogram rapidly diminished in strength and almost entirely disappeared within thirty seconds. The tonus of the segment also diminished. The electrograms persisted and the rate remained constant for from one to three minutes. Records taken five minutes after the drug was given showed muscular activity to be absent. The electrical complexes were greatly diminished in amplitude and the rate was reduced. The electrogram showed very little potential variation in some records. At this time the pulse rate was diminished and the intestinal segment appeared very pallid.

These studies were first carried out in animals in which the mesentery to the intestinal transplants was intact. Therefore, the central nervous system could have influenced the responses, especially through the sympathetic nerves. Later the mesentery was divided thus severing all original connections with the central nervous system. Experiments were repeated using epinephrine, nicotine, atropine and ergotamine and combinations of the latter two. The results corresponded to those obtained before dividing the mesentery. These and numerous observations by various workers on isolated intestinal strips indicate that rhythmic contractions and the waves of altered electrical potential arise within the bowel itself, presumably in the muscle or in the intrinsic nervous systems.

The accuracy of deductions drawn from these experiments depends upon the correctness of our conception of the action of the drugs employed. We assume that the cessation of contraction of the isolated segments produced by epinephrine to be due to a stimulation of the receptive mechanism of the sympathetic system thus increasing sympathetic inhibition. Eserine produced an increase of the strength of muscular contractions but did not alter the rate. Atropine had an opposite effect, that is, it diminished the strength of contractions. As both these drugs are thought to act on the peripheral distribution of the parasympathetics, the former stimulating and the latter paralyzing, and as neither of them greatly altered the electrograms, it suggests that the peripheral parasympathetics have a stimulating influence on the strength of contractions, but do not initiate or greatly influence the rate of the electrogram.

An attempt was made to paralyze the nervous tissue in the segments without greatly damaging the muscle in an effort to determine the site of origin of the waves of altered electrical potential. Ergotamine and atropine were administered simultaneously to paralyze both sympathetic and parasympathetic nerves. These produced an increased amplitude of both myogram and electrogram. Cocaine applied topically to the segment did not greatly alter the electrogram. The local application of nicotine likewise did not interrupt the potential variations. These results suggested the probability of a myogenic origin of the electrogram. However the quantity of drugs used and the method of application did not assure the total paralysis of the intrinsic nervous tissue in the segments. Therefore larger doses of nicotine were given intravenously. This greatly diminishes or interrupts both the electrogram and the myogram and slows the rate but does not alter their relationship to each other when variations are present. Stimulation of the muscle with barium after the use of nicotine greatly increases the strength of muscular contractions with the potential variation remaining almost imperceptible. Were the latter entirely of muscular origin one would expect increased deviation accompanying the accentuated myogram. Berkson (4) working with isolated strips

of rabbit's intestine found that when nicotine was added to the solution in which the tissue was immersed there was an alteration of both mechanical and electrical records followed later by vigorous rhythmic muscular contraction but no manifestation of electrical activity. He suggests "that the rhythmic electrical changes reflect rhythmic pulsations that emanate from the intransic nervous plexus of the intestine."

It is difficult to evaluate the reaction of these segments to pituitary extract. This is known to be a smooth muscle stimulant, yet its use was followed by a supression of both the electrogram and the myogram. This result may have been due to impaired circulation, as was intimated by the marked pallor of the segment after the use of these drugs. Circulatory changes seemed to influence the rate of the electrogram. When the vascularity was increased the rate became slightly more rapid and when the segment became pallid the rate diminished slightly.

The effects of several drugs upon myograms and electrograms obtained from isolated intestinal transplants have been noted. These findings suggest that a rhythmic excitatory process may recur in some intrinsic tissue other than the muscle which initiates muscular contraction. Contractions do not always accompany these excitatory processes but are definitely related to them when they do occur. Existing evidence does not permit a categorical statement as to whether the electrogram is a record mainly of muscular or of intrinsic nervous activity. The balance of evidence however favors the view that the characteristic electrogram represents potential changes associated with activity of the intrinsic nerves.

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THE POSSIBLE MECHANISMS OF CONTRACTING AND PAYING THE OXYGEN DEBT AND THE RÔLE OF LACTIC ACID IN MUSCULAR CONTRACTION[1]

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Although direct evidence of a quantitative relationship between oxygen debt and lactic acid production in man has never been given, there is general agreement with A. V. Hill's hypothesis that the oxygen debt is due to the delayed oxidation of a fraction of the lactic acid produced during the anaerobic processes of muscular activity. After the "revolution" produced in muscle physiology by the discoveries of the last few years, mainly after Lundsgaard's work, and the further observations on men, it seemed to us that the lactic acid mechanism alone is inadequate to explain all the processes which occur in the payment of the oxygen debt, particularly for the following reasons:

a. It has been observed in isolated muscle that most of the lactic acid is produced after the contraction is over, during the first minute of recovery; this does not seem to agree with the fact that the payment of the oxygen debt at the beginning shows no lag even if the exercise is of very short duration.

b. In moderate exercise of long duration in man, involving an oxygen debt of 3 to 4 liters, the changes in lactic acid concentration or in pH of blood are negligible in comparison with the values implied by the assumption of an increment of lactic acid in the body corresponding to the amount of oxygen debt. (See, for the lactic acid concentration in blood following muscular exercise, Owles, 1930; Dill, Edwards, Fölling, Oberg, Pappenheimer and Talbott, 1931; for data on pH, Margaria and Talenti, 1933.)

c. There exist oxidative recovery processes following muscular contraction other than the glycogen resynthesis from lactic acid; Lundsgaard (1931) has demonstrated in muscles poisoned with iodoacetic acid the partial resynthesis of the phosphocreatine in the presence of oxygen and in the absence of any lactic acid formation or removal; Clark, Eggleton and

[1] A preliminary report of this work has been given at the annual meeting of the Federation of American Societies for Experimental Biology in Cincinnati, April, 1933, and the abstract appeared in the Journal of Biological Chemistry, 1933, 100, lxv.

[2] Fellow of the Rockefeller Foundation.

Eggleton (1932) found that heart muscle poisoned with iodoacetate and supplied with oxygen can go on almost indefinitely. Moreover, in the normal muscle only a part of the phosphagen can be restored anaerobically at the expense of the formation of the lactic acid, while a considerable fraction is restored only in the presence of oxygen.

It was our purpose to investigate this problem, particularly in regard to any relation between production and removal of lactic acid and amount and payment of oxygen debt in man.

The behavior of lactic acid. There is no means of measuring directly in man the total lactic acid production or the total amount of lactic acid present in the body at a certain time. The only datum available is the concentration of lactic acid in the blood and we must first make certain whether or not this really represents the total amount of lactic acid present in the body.

The promptness of appearance of lactic acid in muscular exercise as well as the rapid decrease in its concentration as blood passes through an inactive region of the body (Barr, Himwich and Green, 1923), proves that lactate ions diffuse freely between tissues and blood. A free diffusion through the isolated frog's muscles has been observed by Hill (1926) and Evans and Eggleton (see Lovatt Evans, 1930) have found in mammals that within half a minute after a short tetanic contraction, the venous blood coming away from the muscle contains lactic acid at a concentration almost equal to that in the muscle. Thus, except when changes in lactic acid production are so rapid that an equilibrium between the concentrations of lactic acid in tissues and in blood has not been reached, we are justified in assuming the concentration of lactic acid in the blood to be proportional to the total amount of lactic acid in the body.

In preliminary experiments a young athlete, Clapham, ran to exhaustion on a treadmill at 18.7 km. per hour on a 5 per cent grade for $4\frac{1}{2}$ to 6 minutes. The excess lactic acid concentration in the blood, disregarding the values of the first 6 to 8 minutes of recovery, was found to decrease logarithmically according to the formula:

$$L = L_r + 10^{a - bt} \tag{1}$$

where L is the lactic acid concentration at time t, L_r the concentration of lactic acid in the blood at rest, 10^a the extrapolated value of excess lactic acid concentration in the blood at the beginning of recovery and b the velocity constant. The values of the constants a and b were calculated from the experimental values obtained after the first 5 minutes of recovery with the method of least squares, assuming the resting value for lactic acid concentration to be 10 mgm. per 100 cc. of blood. As can be seen in table 1, which gives the values of blood lactic acid concentration in 2 experiments in which the subject ran to exhaustion, the calculated values fit very well

with the experimental ones, the differences being of the same order as the methodical error.[3]

A formula of the type given cannot be applied satisfactorily to the early phase of recovery. This may be due

a. To delayed lactic acid production, though this in isolated frog's muscle has been shown to last less than a minute (see Hartree, 1932).

TABLE 1

Observed and calculated values for lactic acid concentration in the blood of Clapham after running to exhaustion at 18.7 km. per hour on a 5 per cent grade

RECOVERY TIME	LACTIC ACID CONCENTRATION IN ARM BLOOD		
	Observed	Calculated by the formula $L = 10 + 10^{2.113 - 0.0183t}$	Δ
October 14. Duration of performance, 4 minutes			
min.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
½	120		
3¾	107		
5½	108		
7½	104	104.2	+0.2
15½	77.4	77	−0.4
27¼	50.3	50.2	−0.1
56½	24.7	21.7	−3.0
October 28. Duration of performance, 5⅔ minutes			
		$L = 10 + 10^{2.175 - 0.0205t}$	
⅓	108		
5	111		
7	109		
9	108	107.8	−0.2
15	85.4	83.7	−1.7
25	55.1	56	+0.9
61	18.4	18.4	0
100	12	11.5	−0.5
130	10.4	10.3	0.1

b. To the slowness of diffusion of lactic acid from the tissues to blood, and

c. To the lower O_2 tension in the tissues in the first part of recovery, due to failure of the O_2 supply to keep pace with the greater oxygen consumption.

This lag in the disappearance of lactic acid is greater the shorter the

[3] The method used for determining lactic acid was that of Friedemann, Cotonio and Schaffer (1927); the blood was collected from an antecubital vein unless otherwise noted.

exercise and the higher the degree of exhaustion. In fact if the exercise is hard and short enough a rise in blood lactic acid concentration may be observed in the first part of recovery (Hill, Long and Lupton, 1924; also see protocol 9). Margaria and Talenti (1933) have found under such conditions an associated increase in hydrogen ion concentration. The lag is nearly eliminated if the period of exercise is made to last longer, say 10 minutes, which shows that factors *a* or *b* or both are the most important in determining such a lag.

The fact that the removal of lactic acid from the body is a logarithmic function of time shows that the rate of removal of the excess lactic acid is proportional to the concentration of the excess lactic acid itself. This fits the conception that the removal of lactic acid is due to the combustion of a fraction of it. We must assume, however, that after the first few minutes of recovery the tissue O_2 tension remains practically constant and the physicochemical changes in the body do not interfere with the process of lactic acid oxidation.

The payment of the oxygen debt, on the other hand, has never been shown to be a logarithmic or any other simple function of the time in spite of efforts made in this direction. Hill, Long and Lupton (1924) affirm that "there are clearly two factors at work, one of which accounts for the initial rapid fall in the oxygen intake on the cessation of exercise, the other for the prolonged remainder of recovery occurring after extended or severe exertion. . . . . Each process is exponential in character—one is rapid; one, however, is slow. Moderate exercise is followed to a preponderant degree by recovery of the rapid type." These authors attribute the first and rapid phase of recovery to the oxidative removal of lactic acid in the muscles where it was formed, while the second and prolonged phase represents the oxidative removal of lactic acid which has had time to escape by diffusion from the muscles.

If this hypothesis were true we ought to find a close proportionality between oxygen debt and lactic acid concentration in the blood, provided that the exercise has been long enough for complete diffusion of lactic acid to have taken place between muscles and blood at the end of the exercise.

Method. We have performed a series of experiments on the same subject in which the exercise lasted 10 minutes. We assume that in this time and for exercise not involving the maximum metabolic rate, equilibrium is reached as regards diffusion of lactic acid from the muscles at work into the blood stream and from this into the tissues which were not producing lactic acid. This assumption has been proved very nearly correct. After such a period of work, provided a steady state has been reached, the concentration of lactic acid in the blood decreases without showing an appreciable lag from the end of the exercise.

Evidence for this statement is given also by protocol 7 in which the

subject ran for 10 minutes at 14 km. per hour on a 2.5 per cent grade. Samples of blood from the femoral vein, and an arm vein and from the femoral artery were taken at various periods after exercise. In spite of the fact that the exercise was very strenuous involving a metabolic rate very near the maximum, there was but little difference in lactic acid concentration or pH of blood samples taken simultaneously from the various sources. Figure 1 shows the phenomenon; the values for lactic acid concentration are the same for arterial and arm vein blood, and only slightly higher in blood from the femoral vein during the first minutes of recovery.

Various amounts of oxygen debt were produced by varying the intensity of the exercise, which consisted of walking or running on a motor-driven treadmill at various speeds and at various grades.

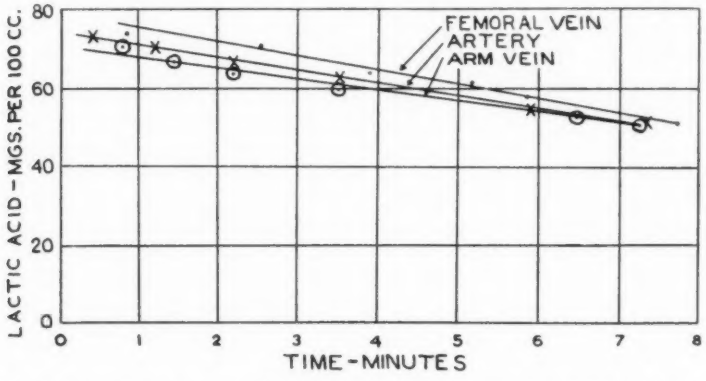


Fig. 1. Lactic acid concentrations of arterial, (×), femoral vein (·), and arm vein blood (⊙) in recovery, as a function of time, in Clapham after a 10 minute run at 14 km. per hour on a 3.8 per cent grade.

There is some trouble in measuring the extra oxygen consumption as an effect of previous muscular exercise (we shall not use temporarily the term "oxygen debt" which specifies a mechanism as to the use of the oxygen) as the oxygen consumption after a considerable amount of work does not come back, even in two hours, to the pre-work values. For example, the basal O_2 consumption for the subject Clapham was 240 cc. per minute; this value was reached one-half hour after a 10 minute run at 7.4 km. per hour, while after harder exercise his oxygen consumption per minute was still as high as 270 cc. two hours after the end of work. The resting oxygen consumption then is merely a matter of subjective appreciation. So, following A. V. Hill's procedure, in our first calculations we assumed as resting the O_2 consumption one and one-half hour after the end of the work.

Relation between lactic acid production and oxygen debt, as calculated after

A. V. Hill. Plotting in a coördinate system the concentration of lactic acid in the blood at the beginning of recovery against the after-work extra oxygen consumption so calculated, we obtain the curve represented in figure 2.

For the contraction of an excess oxygen consumption during recovery up to 3 or 4 liters we see no appreciable increase of blood lactic acid. For extra oxygen consumption of 6 liters or more, the concentration of lactic acid in the blood is a linear function of the extra oxygen consumption. It seems that the most obvious way of explaining this behavior is to admit

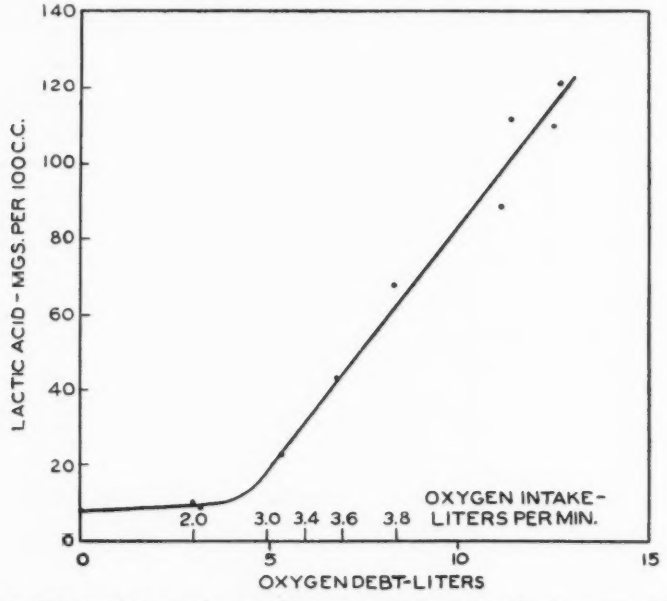
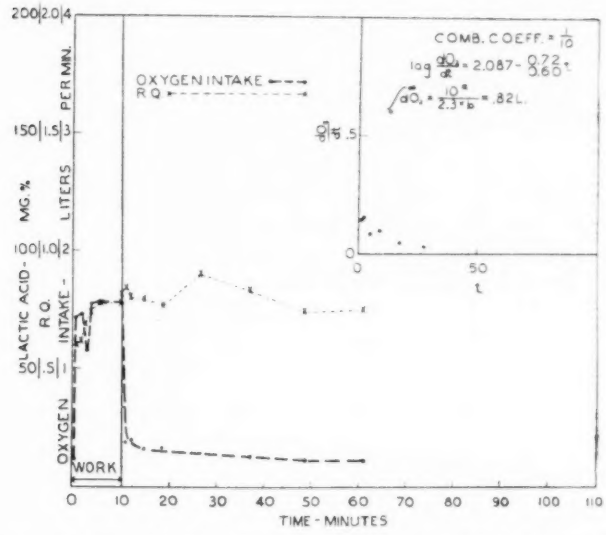
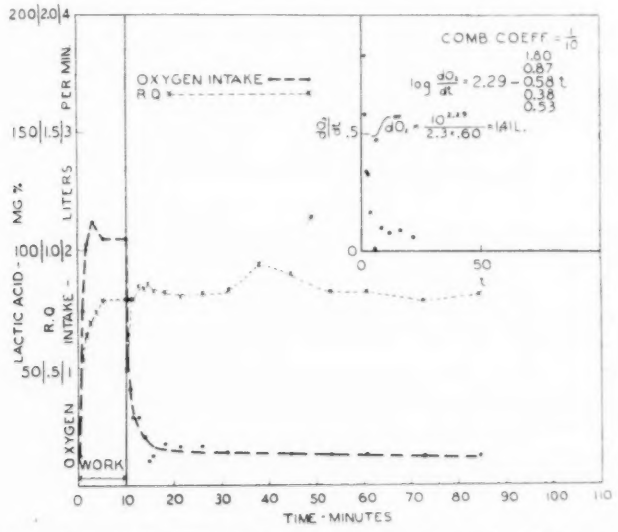


Fig. 2. Relation between lactic acid concentration in the blood, oxygen debt as calculated after A. V. Hill, and metabolic rate, at steady state of exercise. Duration of exercise was 10 minutes in each case.

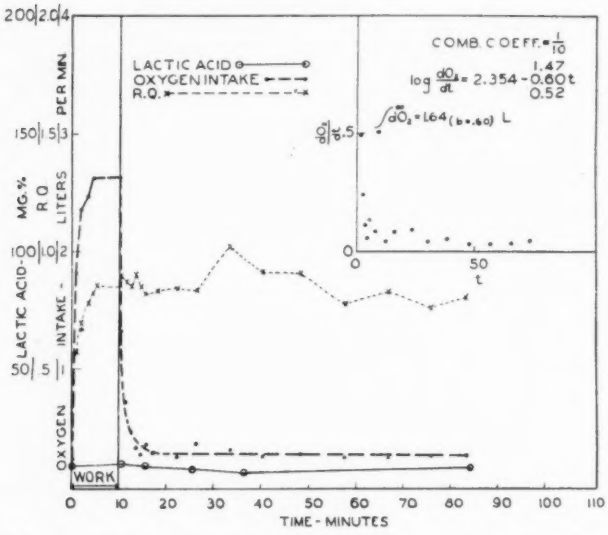
that when the excess oxygen is less than 3 liters the lactic acid mechanism is not involved but some other process, at present unknown. The lactic acid mechanism starts coming into play when the extra oxygen consumption is 3 to 4 liters, while for increasing the extra oxygen consumption over 4 liters it seems that only the lactic acid mechanism is involved. That there is no extra accumulation of lactic acid, even localized in the muscles, corresponding to small amounts of extra oxygen consumption after work is also proved by the behavior of the R. Q. which does not increase in the first stages of recovery more than can be accounted for by the retention of CO_2 at the beginning of exercise (see protocols 1 to 4).



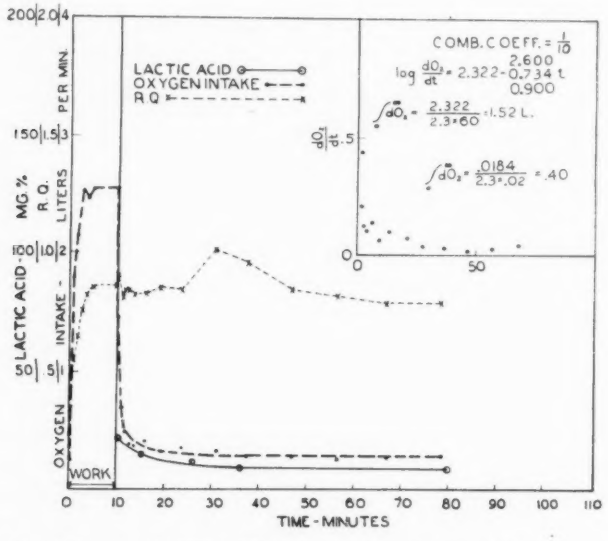
Protocol 1. February 1, 1933. Clapham walking at a speed of 7.5 km. per hour for 10 minutes. Corner figure: Oxygen consumption per minute less the minimum basal oxygen consumption. The values for the speed constants of the alactacid process in this and the following protocols are given for the single experimental data in order of time, as well as the value of the alactacid oxygen debt ($\int_0^\infty dO_2$).



Protocol 2. January 25, 1933. Clapham running at a speed of 9.3 km. per hour for 10 minutes. Corner figure: Oxygen consumption per minute less the minimum basal oxygen consumption.


LACTIC ACID
OXYGEN INTAKE
R.Q.
COMB. COEFF. = 1/10
log dO2/dt = 2.354 - 1.47/0.52 0.60t
∫dO2 = 1.64 (b = .60) L
LACTIC ACID - OXYGEN INTAKE - R.Q. - MG.% - LITERS PER MIN
WORK
TIME - MINUTES

Protocol 3. January 27, 1933. Clapham running at a speed of 11.3 km. per hour for 10 minutes. In the inserted figure in protocols 3 to 8: oxygen consumption per minute less the minimum basal oxygen consumption and less the amount attributable to the combustion of the excess lactic acid on the assumption of a combustion coefficient of $\frac{1}{10}$.


LACTIC ACID
OXYGEN INTAKE
R.Q.
COMB. COEFF. = 1/10
log dO2/dt = 2.322 - 2,600/0.900 0.734 t
∫dO2 = 2.322/2.3*60 = 1.52 L.
∫dO2 = .0184/2.3*.02 = .40
LACTIC ACID - OXYGEN INTAKE - R.Q. - MG.% - LITERS PER MIN
WORK
TIME - MINUTES

Protocol 4. February 15, 1933. Clapham running at a speed of 11.3 km. per hour on a 1 per cent grade for 10 minutes.

From the slope of the curve in the later and linear part we can see for this subject that 1 liter of extra oxygen consumption corresponds to a concentration change of 12.8 mgm. of lactic acid in 100 cc. of blood. Assuming a uniform distribution of lactic acid in the body, with water contents of 80 per cent in the blood and of 72 per cent in the body as a whole, the body weight of the subject being 61.2 kgm., 12.8 mgm. of lactic acid in 100 cc. of blood would correspond to 7.0 grams in the body. This amount is removed in the body by 1 liter of oxygen. Since in the combustion of lactic acid, 3 mols. of O_2 are used for 1 mol. of lactic acid, assuming that the increment in extra oxygen consumption after work in the conditions referred to is only used up in the combustion of the lactic acid, 1 liter of oxygen must be used for the combustion of 1.34 grams of lactic acid, the remaining being resynthesized as glycogen. The ratio between lactic acid burned and total lactic acid removed is $\frac{1.34}{7.0} = \frac{1}{5.2}$. This value corresponds to the lowest given by A. V. Hill and by Meyerhof for the recovery in isolated frog's muscle.

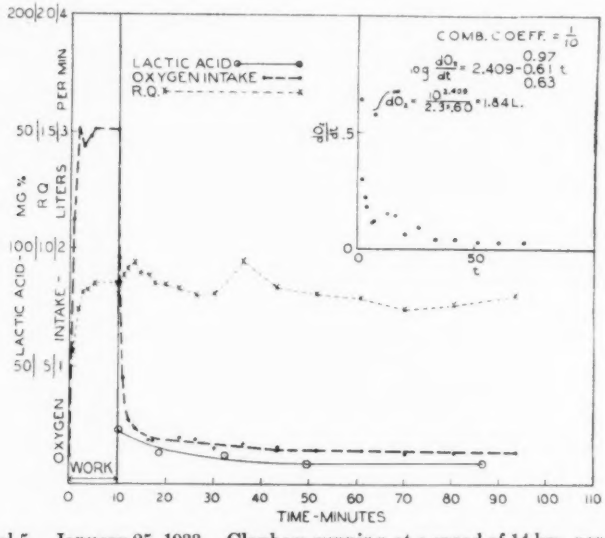
As we shall see later, the assumption that the extra oxygen consumption is only used in the combustion of lactic acid is probably incorrect, as other oxidative processes are going on. The value $\frac{1}{5.2}$ given above is lower than the average obtained by Hill and Meyerhof. This is due to the fact that in our calculation we avoided the error of using the whole of the extra oxygen consumption for comparison with the whole lactic acid removed, but employed instead the increment of the extra oxygen consumption for comparison with the increment of lactic acid on the straight portion of the curve.

Figure 2 shows also lactic acid concentration in blood as a function of metabolic rate. The assumption that increase in blood lactic acid is proportional to increase in metabolic rate is not supported by the facts. We see that extra lactic acid formation does not occur except in severe work. Also the lactic acid produced does not account for all the extra oxygen consumed after work, particularly for easy work. In easy work there must certainly be an oxygen debt too, which may be accounted for by the fact that the metabolic rate during work does not reach its steady state immediately, but after an initial period during which the oxygen consumption is lower than the corresponding amount of work. We may then distinguish two mechanisms of contracting O_2 debt, the one due to the lactic acid, which we shall call the "lactacid" mechanism, and an "alactacid" one where there is no apparent lactic acid formation. The lactic acid mechanism in this subject, running on a treadmill, comes into play at an O_2 consumption of about 2.4 liters per minute which is 60 per cent of his maximum metabolic rate.

The alactacid mechanism occurs then far more frequently in the ordinary

conditions of life than the lactacid mechanism. This fact, together with the fact that, as we shall see later, the alactacid oxygen debt is paid at a much faster rate than the lactacid oxygen debt, raises the importance of the alactacid mechanism of contracting the oxygen debt to a primary degree. The lactacid mechanism has to be considered more like a mechanism of emergency, though its capacity is greater than that of the alactacid O_2 debt mechanism.

Rôle of lactic acid in muscular contraction. It appears from figure 2 that up to a considerable rate of work, not exceeding two thirds of the maximum aerobic rate of work, no lactic acid excess appears in the blood, and presumably in the muscles, for the case of the athletic subject investigated. Does that mean that no excess lactic acid is formed in the muscles as a consequence of that amount of work?

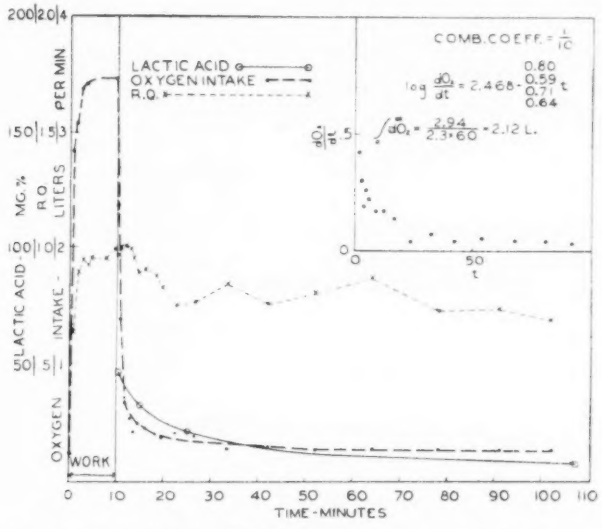


Protocol 5. January 25, 1933. Clapham running at a speed of 14 km. per hour for 10 minutes.

The concentration of lactic acid in the muscles depends upon an equilibrium between lactic acid formation and lactic acid removal: there is no doubt that, if the rate of lactic acid formation increases without a corresponding increase in the rate of its removal, a new equilibrium will be set up at a higher lactic acid concentration. The removal of lactic acid, being due to the oxidation of a fraction of the lactic acid itself, depends upon the oxygen tension in the muscles: it is highly improbable and contrary to the usual findings that the oxygen tension is higher in the working muscles

than in the ones at rest; therefore, unless the lactic acid removal in these conditions is due to another mechanism which goes at a faster rate than the mechanism of removal of the lactic acid during recovery for a lactacid oxygen debt, for which we have no evidence, it follows that muscular work at such a rate is accomplished without any lactic acid formation.

The slowness of the removal of lactic acid during recovery may be due either *a*, to the fact that the speed of the oxidation of the lactic acid is low, or *b*, to the speed of the reaction lactic acid → glycogen being low. In case of *a* we have to exclude the possibility of any important part played by the lactic acid in normal aerobic muscular contractions, because, on this

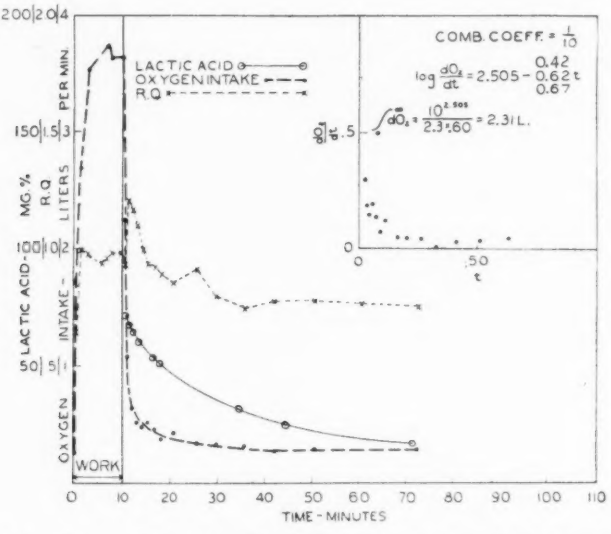


Protocol 6. February 1, 1933. Clapham running at a speed of 14 km. per hour on a 2.5 per cent grade for 10 minutes.

assumption, we would reach in moderate exercise excessively high levels of lactic acid in the body.

For example, assuming that the work is all performed at the expense of the combustion of lactic acid and that no lactic acid is resynthesized to glycogen during work, for the subject Clapham working in a steady state at a metabolic rate of 2 liters of oxygen per minute, a calculation, on the basis that the speed constant for the removal of lactic acid is 0.02, leads to a value of increased lactic acid concentration in the blood of 5 grams per liter. Actually, for such a metabolic rate, we do not observe any increase in lactic acid. On the other hand evidence that oxidation of lactic acid is a slow process is found in the facts *a*, that at the beginning of recovery when

the oxygen consumption is still very high, the decrease in concentration of lactic acid is no greater than in the later period of recovery, and *b*, that for all the period of recovery the combustion of lactic acid accounts only for a small fraction of the total oxygen consumption. We would have expected that, given a great excess of lactic acid in the body, there ought to be at least a proportionality between oxygen consumption and lactic acid disappearance. Moreover, as we shall see later, the speed of removal of lactic acid is affected by the oxygen tension in the inspired air and therefore in the tissues, which shows that the slowness of the reaction is due to the



Protocol 7. February 23, 1933. Clapham running at a speed of 14 km. per hour on a 3.8 per cent grade for 10 minutes.

oxidation of the lactic acid and not to the reaction lactic acid → glycogen which must be independent of the oxygen tension.

It is justifiable, therefore, until direct data on the speed of oxidation of lactic acid in the body are available, to assume as more probable the hypothesis that the slowness of removing lactic acid is due to the slowness of the process of its oxidation.

This interpretation does not, of course, exclude the possibility of the interpretation given by A. V. Hill in 1924, i.e., that the lactic acid, though being formed at all rates of muscular work, does not escape out of the muscles below a certain metabolic rate because "it is hemmed in by a zone in which oxygen supply is adequate, and through which it cannot pass: only

when some regions of the muscles become oxygen-free can lactic acid pass through them and escape into the capillaries." We think, however, that this hypothesis can hardly be reconciled with the following facts: *a*, no sign of the presence or removal of any acid in the body can be detected from the behavior of the R. Q. up to an after-work oxygen consumption of 4 liters. The period required for consuming this amount of oxygen is long enough for such phenomena to be observed, if present; *b*, the time required for the removal of lactic acid from the blood is certainly much greater than that which could be accounted for by simple diffusion from blood to the muscles. This requires the assumption that lactic acid diffused into the blood cannot be utilized by the muscles again, an assumption for which we have no evidence.

Oxygen consumption due to the lactic acid removal. If the lactic acid concentration of the blood decreases logarithmically, its speed of removal decreases also logarithmically. Thus we have, from (1),

$$-\frac{dL}{dt} = 2.3 \times b \times 10^{a-bt} \tag{2}$$

Assuming the total amount of lactic acid in the body uniformly distributed per amount of water, then for our subject, 1 mgm. of lactic acid concentration in 100 cc. of blood corresponds to 0.55 gram of lactic acid: and assuming a combustion coefficient of lactic acid of $\frac{1}{5}$, i.e., $-\frac{dL}{dO_2} = 6.7$, where L is expressed in grams in the whole body and O_2 in liters, and substituting in (2) we have

$$\frac{dO_2}{dt} = \frac{2.3 \times 0.55}{6.7} \times b \times 10^{a-bt} \tag{3}$$

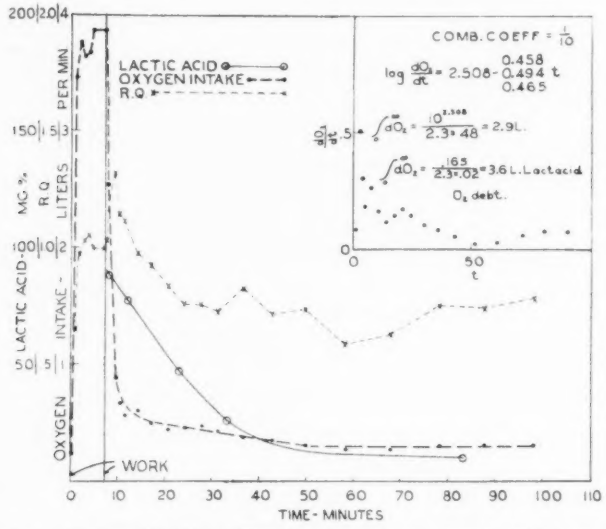
which expresses the oxygen consumption due to the removal of the lactic acid on the assumption above. The amount of oxygen debt due to this mechanism will be given by

$$\int_0^{\infty} dO_2 = \frac{0.55}{6.7} \times 10^a \tag{4}$$

Subtracting from the values of the oxygen consumption after work the amount due to the basal metabolic rate (0.24 liter per min.) and also the amount due to the lactacid oxygen debt (if any) we obtain curves all more similar to each other. The value of the velocity constant b has been assumed in these calculations to be 0.02.

The fact that the curves of the remaining oxygen consumption become all more similar to each other is particularly interesting as it was known, after A. V. Hill's work, that the payment of small oxygen debts goes on at a

relatively much faster rate than the payment of large oxygen debts. This fits in with the hypothesis that the lactacid payment of the oxygen debt is a slow process and that such a mechanism only enters into play when large amounts of oxygen debt are required. We must remember that the speed of the payment of the lactacid oxygen debt is the same as the speed of disappearance of the lactic acid from the blood; the velocity constant is for both processes about 0.02, which means that 50 per cent of the reaction is not reached until after 15 minutes and that it takes 1 hour to reach 94 per cent of completion.


LACTIC ACID
OXYGEN INTAKE
R.Q.
COMB. COEFF = 1/10
log dO2/dt = 2.508 - 0.494 t
0.458
0.465
∫dO2 = 10^2.508 / 2.3·.48 = 2.9L.
∫dO2 = 10^1.65 / 2.3·.02 = 3.6 L. Lactacid O2 debt.
LACTIC ACID - MG. % OXYGEN INTAKE - LITERS PER MIN. R.Q.
200 20 4
150 15 3
100 10 2
50 5 1
WORK
0 10 20 30 40 50 60 70 80 90 100 110
TIME - MINUTES

Protocol 8. January 27, 1933. Clapham running at a speed of 18.6 km. per hour for 7 minutes.

The oxygen consumption curve from which the minimum resting O_2 consumption and the amount attributable to the lactic acid removal have been subtracted. The curve of the oxygen consumption in recovery less the minimum resting oxygen consumption and the oxygen used in paying the lactacid debt seems to be the sum of two curves, corresponding to two processes occurring at very different rates. The composite curve falls rapidly at the beginning of recovery, a process lasting only 3 to 4 minutes, and then falls slowly and rather irregularly, a process lasting perhaps one-half hour in mild work and two hours or more after strenuous exercise.

The possibility arises that the first rapid fall of the oxygen consumption is due to the presence in the body of an oxygen reserve mechanism. Such an oxygen reserve could be the expression of the difference of saturation of

the hemoglobin between rest and work, particularly for the hemoglobin contained in the working muscles. In Henderson's charts (1928) we see that the difference in oxygen saturation of venous blood between rest and work at an oxygen consumption of 1750 cc. per minute in a non-athletic man corresponds to 40 cc. per liter of blood. Assuming the volume of venous blood is 1.5 liter, only 60 cc. of oxygen could be attributed to this mechanism. Taking into account also the difference in saturation of the arterial blood and of the muscle hemoglobin, we believe that we can safely state that the total amount of oxygen debt attributable to this mechanism cannot exceed 100 cc., which is negligible for the present purposes.

The alactacid mechanism of O_2 debt. In mild exercise we may consider the curve of the oxygen consumption in recovery less the minimum resting O_2 consumption without lactic acid formation as resulting from the summation of two processes. Extrapolating to zero recovery time the slow process, the difference between the summated curve and that corresponding to this slow process will permit an analysis of the fast process. The curve that we obtain in this way is within the limits of experimental error, of the same logarithmic type as the curve of the oxygen consumption due to lactic acid removal, only the speed of this process is much greater. As for the lactic acid mechanism, this function is defined by

$$\log \frac{dO_2}{dt} = a_1 - b_1 t \tag{5}$$

b_1, the velocity constant, is easily calculated knowing the value of a_1, which is defined by $\frac{dO_2}{dt} = 10^a$ for $t = O$.

The fact that this process has the characteristic of logarithmic decrement is good evidence for attributing to it the significance of an oxygen debt payment. This portion of the debt may be called "alactacid" because it takes place without apparent extra lactic acid formation. The more reliable value of b_1 is obtained at t = about 2 minutes, because for very small values of t the experimental error may be very considerable, and for higher values of t errors due to incorrect extrapolation of the slow function may become considerable. The values for b_1, so calculated for the different times, are given in the protocols: the more reliable value is about 0.60.

This alactacid mechanism of paying an oxygen debt is much more convenient than the lactacid mechanism as far as the speed of payment is concerned. This speed is 30 times greater, taking only half a minute to pay 50 per cent of the alactacid debt, while the payment is practically complete (98.5 per cent) in three minutes.

The total amount of oxygen debt contracted by this mechanism is defined by

$$\int_0^{\infty} dO_2 = \frac{10^{a_1}}{2.3 \times b_1} \tag{6}$$

The coefficient of combustion of lactic acid. In experiments where a considerable amount of lactic acid was formed curves may be derived showing the residual oxygen consumption after subtraction of the minimum resting oxygen consumption and of the oxygen consumption due to the payment of the lactacid oxygen debt on the assumption of a combustion coefficient for lactic acid of $\frac{1}{5}$. These curves are dissimilar in two respects from corresponding curves obtained in mild exercise without lactic acid formation. One dissimilarity is found in the shape of the curve corresponding to the slow process. Values for O_2 consumption in this process are smaller in mild exercise. The curve may be flat for the first 1 or 2 hours of recovery and in some cases negative values for O_1 consumption are indicated. Another dissimilarity is observed in the speed constants. Those calculated in experiments where excess lactic acid formation occurs are about one-half the magnitude of corresponding values derived in the absence of apparent lactic acid formation.

If we calculate such curves on the assumption of a combustion coefficient for lactic acid of $\frac{1}{1.6}$ the resulting curves appear to be more nearly the same type as the corresponding curves for mild exercise. Values for oxygen consumption are always positive and the oxygen consumption for the slow process tends to decrease with time as in milder exercise. Also uniform values for the speed constants of alactacid recovery are derived by this calculation whether or not excess lactic formation occurs. Finally, in a given experiment, the values for speed constants calculated at different times agree better with each other (see protocol 9). Therefore, it seems to us justifiable to assume as more nearly correct a value of $\frac{1}{1.6}$ for the combustion coefficient of lactic acid.

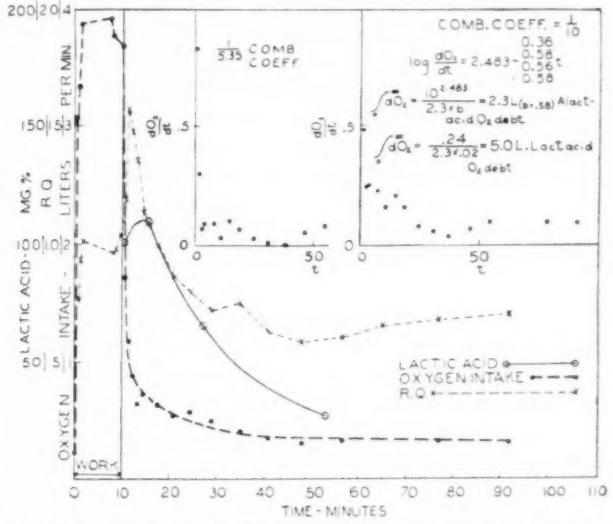
The fact that a combustion coefficient of $\frac{1}{5.2}$ was found from the data from figure 1 and $\frac{1}{5}$ from the data of Hill and Meyerhof may be explained by the fact that in these cases the computation of the oxygen involved not only the oxygen removal of lactic acid but also the oxygen used in the slow process, which may amount to nearly the same value.

Also from a theoretical point of view the value of $\frac{1}{1.6}$ as the combustion coefficient of lactic acid seems more probable than the value $\frac{1}{5}$. If the latter value is correct, the heat of combustion of lactic acid as lactate being 3,501 calories per gram of lactic acid and the heat of transformation of 1 g. glycogen to 1 g. lactic acid, as lactate in the muscles, 340 calories, the efficiency of resynthesis of glycogen from lactic acid comes out to be less than 40 per cent. Now the mechanical efficiency of muscular work performed anaerobically at the expense of glycogen breakdown to lactic acid may be not less than 30 per cent. It follows that the efficiency of glycogen breakdown into lactic acid and the transformation of this chemical energy into mechanical has an efficiency of over 75 per cent. It is hardly believable that this process takes place at a higher efficiency than the proc-

ess of resynthesis of glycogen from lactic acid. This, being a pure chemical process not involving transformation into other kinds of energy, occurs presumably at a very high efficiency.

On the assumption of a combustion coefficient of lactic acid of $\frac{1}{10}$ the efficiency of resynthesis of glycogen from lactic acid would be about 87 per cent.

The increase in basal metabolism after work. The remaining oxygen consumption does not seem to have anything to do with a real oxygen debt as ordinarily defined. In other words, the oxidative processes which account



Protocol 9. February 15, 1933. Clapham running at a speed of 18.6 km. per hour for 10 minutes. Corner figures: Oxygen consumption per minute less the minimum basal oxygen consumption and less the amount attributable to the combustion of the excess lactic acid on the assumption of a combustion coefficient of $\frac{1}{10}$, and of $\frac{1}{5.35}$.

for such an amount of oxygen do not appear to provide the energy for the reconstitution of substances which broke down in consequence of the muscular contraction and whose anaerobic breakdown furnished directly or indirectly the energy developed in the muscular contraction. This conception is supported by the fact that this oxygen consumption takes such a long time to reach the zero value; it may still be 10 to 20 per cent of the minimum basal oxygen consumption, 1 or 2 hours after the end of the work, when quantitatively important properties of the blood have returned to normal. The hypothesis of an increase in the basal metabolism after work was advanced by Hill, Long and Lupton (1924) on the basis of

their findings and those of Benedict and Cathcart (1913) of an increased resting oxygen consumption several hours after the end of severe muscular exercise. The former authors assumed a constant value for the increase in metabolism for all the period of recovery, a value that they deduced from measurements of the oxygen consumption 2 or 3 hours after the end of work.

It seems to us that our calculation indicating that the oxygen used in this slow process decreases uniformly explains the phenomenon in a more satisfactory way than does the assumption of a constant increase of oxygen intake for this process. Since the phenomenon is due to muscular work and continues in evidence for several hours of recovery, it appears likely that it will be more pronounced in the early phase of recovery.

Data on the amount of oxygen consumption due to this mechanism are given in the protocols. After exercise of 10 minutes' duration requiring an oxygen consumption of 1550 cc. per minute, in the first 5 minutes of recovery such extra rest oxygen consumption may be 120 cc. per minute (50 per cent of the minimum resting oxygen consumption). It may be 250 cc. per minute (over 100 per cent of the basal oxygen consumption) for harder work. The time required for attaining the minimum resting oxygen consumption is greater the more severe and prolonged the work.

The oxygen debt as computed by our method may differ greatly from the value obtained by A. V. Hill's method, which does not take into account all the increase in oxygen consumption after work. Thus, in the 10-minute run at 18.7 km. per hour the oxygen debt as calculated after A. V. Hill for $1\frac{1}{2}$ hours after the end of work was 12.5 liters. According to our calculations the lactacid oxygen debt was 5 liters, the alactacid one 2.3 liters and the oxygen used in that period of time attributable to an increased rest oxygen consumption amounted to 10.6 liters. The amount of oxygen debt as calculated following Hill's procedure is 70 per cent higher than our value. In the 7.4 km. per hour run our value for oxygen debt is 0.82 liter while following A. V. Hill's procedure it is 2.55, three times as great.

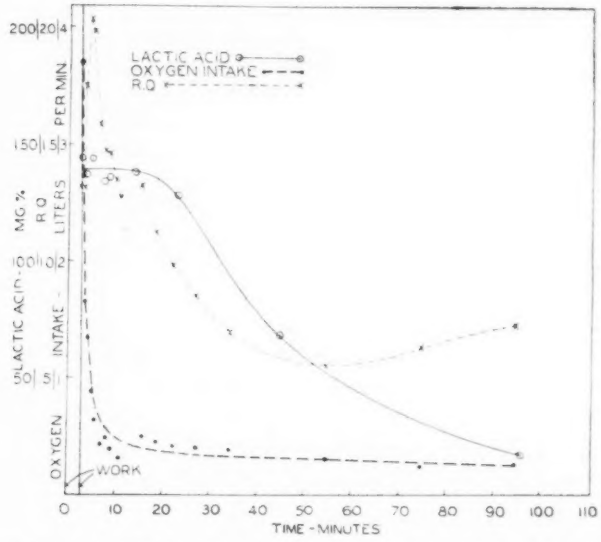
This increased oxygen consumption may account for the oscillations and irregularities of the values for oxygen consumption after work. They were not seen in this subject in determinations of the basal metabolism carried on for several hours,[4] nor are they detectable in the curves of lactic acid removal from the blood and therefore in the corresponding oxygen consumption.

The delayed lactic acid appearance in arm venous blood. We mentioned before that the curve showing lactic acid concentration in blood during recovery in the first few minutes deviates from the curve calculated on the

[4] In 9 determinations of the basal metabolism performed on the subject Clapham from 8:30 a.m. until 12:30 p.m. the average oxygen consumption came out to be 0.240 liter per minute with a probable deviation from this value of ± 0.004.

data of a later period. This phenomenon, however, only follows high degrees of exhaustion, and it is greater the shorter the exercise. The duration of this delay in various experiments on Clapham was about 6 to 8 minutes, while in another subject, only capable of a poor performance the curve of the lactic acid removal took 25 to 30 minutes to reach the normal logarithmic shape (see protocol 10).

Of the possible explanations, pointed out on p. 691, the low oxygen tension in the tissues at the beginning of recovery does not seem to affect the speed of removal of lactic acid to such an extent as to explain this phe-



Protocol 10. February 2, 1933. Shaeron running at a speed of 14 km. per hour for 3 minutes.

nomenon. On the other hand the delayed lactic acid production in isolated frog muscle at 20° lasts a very short time after contraction, and is complete in a minute or so. The delayed lactic acid equilibrium in blood then must be attributed to the slowness of diffusion of the lactic acid from the muscles to the blood stream and into idle tissues.

Amounts of the lactacid and alactacid oxygen debts and their significance. If we plot the amount of lactacid and alactacid oxygen debts as calculated on the assumption of a combustion coefficient for lactic acid of $\frac{1}{1}\frac{1}{0}$ against the metabolic rate, for the experiments lasting 10 minutes, we obtain the curves represented in figure 3.

The alactacid oxygen debt increases linearly with increase in metabolic

rate to reach a maximum of about 2.5 liters at the maximum metabolic rate, 3.6 liters of oxygen per minute in excess of the resting value. So the alactacid oxygen debt amounts in this subject to 700 cc. for an increase in metabolism of 1 liter of oxygen. It does not seem to be related directly to the intensity of work performed since it does not increase as an increasing proportion of the work is carried on by anaerobic processes.

In moderate work the alactacid oxygen debt accounts for nearly all the lag in oxygen consumption at the beginning of work before a steady state is established.

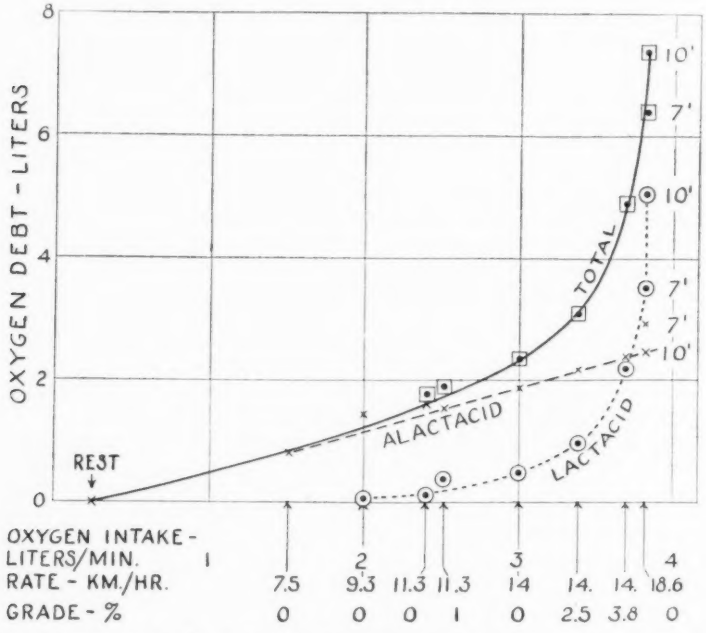


Fig. 3. Amounts of alactacid and of lactacid oxygen debts as a function of the metabolic rate: subject Clapham: from protocols 1 to 9.

The lactacid oxygen debt in Clapham is not appreciable until the oxygen intake is about 2.5 liters of oxygen per minute. It increases slowly at first, more rapidly later and the curve becomes vertical at the point corresponding to the maximum metabolic rate. In this region it increases with increasing amount of work performed, which shows that at the highest metabolic rates an increase in oxygen debt is made only at the expense of the lactacid mechanism.

The alactacid oxygen debt is very probably the expression of an oxidative

process in which the energy liberated is spent in repaying the amount of energy set free anaerobically during the muscular contraction, i.e., it is a true oxygen debt in the meaning given by A. V. Hill to this term.

We do not know the identity of the substances oxidized nor the nature of the anaerobic process that requires this latter oxidative process. However, the more important anaerobic change in muscle during contraction, excluding the lactic acid formation, is the splitting of phosphagen into creatine and H_3PO_4 and we know that the reverse process requires, at least in part, the presence of an oxidative process. It seems, therefore, justifiable to advance the hypothesis that the resynthesis of the phosphagen is the process which absorbs the energy developed in the oxidative processes attributable to the alactacid oxygen debt payment. This hypothesis could be checked knowing the amount of oxygen which is required in the resynthesis of the phosphagen, and the amount of phosphagen in the muscles at work.

During rest a certain equilibrium must exist in the muscles between phosphagen and its products of scission. Since phosphagen breakdown accompanies muscular contraction, the steady state of exercise is associated with a new equilibrium in which the amount of phosphagen will be less and the amount of the products of its scission will be greater than at rest. At the end of the work there will be a tendency toward reaching the rest equilibrium state again, and this will go at the expense of the oxidative processes responsible for the alactacid oxygen debt.

This all fits with the findings that an oxygen debt is found also at the lowest rates of work and also with the approximate linear relation between the rate of oxygen consumption and alactacid oxygen debt.

Eggleton (1930) has found that the creatine equilibrium concentrations for resting and for fatigued muscle are respectively 0.005 and 0.023 molar. Since 11,000 calories are evolved per gram molecule of phosphagen hydrolyzed (Meyerhof and Lohmann, 1928) the difference in phosphagen content between fatigued and resting muscle would account for 200 calories per kilogram of muscle, which corresponds to about 0.043 liter of oxygen. On the hypothesis that the alactacid oxygen debt accounts for the resynthesis of phosphagen from its components, 2.5 liters correspond to the exhaustion of about 60 kgm. of muscle. Taking into account that the efficiency of this process may lie, presumably, between 40 and 80 per cent, we reach a figure of about the expected order of magnitude.

On the basis that during muscular contraction phosphagen splits down into H_3PO_4 and creatine and that this may account for the alactacid oxygen debt, we have measured inorganic phosphorus and creatine contents of blood after strenuous work. There is quantitatively little change in the content of creatine, and an increase in the concentration of inorganic phosphorus from about 0.0018 mol. in rest to about 0.0025 after work: this

change is much too small to account for the amount of phosphagen split down, as calculated from the oxygen debt. However, this may be due either to the esterification of the H_3PO_4, originating from the phosphagen, with the glucose, a process which is known to happen, or to the fact that phosphate is practically indiffusible through the muscle membrane (G. Eggleton, 1933); the smallness of the creatine increase in the blood may be explained by its failure also to diffuse out of the muscle cells.

We venture no hypothesis as to the nature of the substances oxidized in payment of the oxygen debt; we have no reason to suppose that they are substances other than those usually burned during other activities of the tissues.

As far as the lactacid oxygen debt is concerned, there is no doubt, after the work of Hill and Meyerhof, that this accounts for the energy of resynthesis of the glycogen from the lactic acid. However, we have no direct evidence for supposing that the destiny of such oxygen is to burn part of the lactic acid itself. The slowness of the removal of the lactic acid may be due to the fact that the resynthesis of the lactic acid into glycogen is a slow process, or to the fact that the oxidative processes furnishing the energy for such a resynthesis are slow. In the latter case we have to assume that the resynthesis of the glycogen cannot take place by oxidation of the usual fuel, because these oxidative processes may go at a much faster rate. This is evident from the behavior of the payment of the alactacid oxygen debt and from the oxidative processes taking place when muscular work is performed aerobically at the highest metabolic rate.

That this really is so is very probable, in view of the influence of the oxygen content in the inspired air on the speed constant of the removal of lactic acid. Then we must conclude that the speed of disappearance of lactic acid is slow because the combustion of the lactic acid is a slow process, and that the resynthesis of glycogen from lactic acid can only occur at the expense of the oxidation of the lactic acid itself.

A fraction of the oxygen debt being due to lactic acid formation, we can increase the capacity of oxygen debt by increasing the capacity of the body for lactic acid. Meyerhof showed that the lactic acid concentration in the exhausted isolated muscle is much greater if the muscle is kept in an alkaline medium.

Also in man the values of blood lactic acid following exhaustion are much higher if the man started the work with higher alkali reserve (see Dill, Edwards and Talbott, 1932). This may mean that we may be able to increase the capacity for work by increasing the alkali reserve.

We performed two experiments on Clapham in which three hours before the performance of work he took 20 grams of sodium bicarbonate: the exercise consisted in running on the treadmill at 18.7 km. per hour on a 5 per cent grade. In table 2 the two experiments are compared with two

other experiments performed in the same conditions but without previous ingestion of alkali.

It seems from these data that really, together with a higher lactic acid concentration in the blood, the performance was better. No appreciable change can be seen in the total O_2 consumption after exercise; however this measurement is subject to large sources of error, certainly greater than the variation expected, which is 1 liter of oxygen for every 24 mg. of increase in lactic acid concentration in the blood.

Calculations of the lactacid oxygen debt, as described in this paper, on the experiments with ingestion of $NHCO_3$ cannot bring evidence for an increase of the lactacid oxygen debt, as they are made on the assumption that the lactacid oxygen debt is proportional to the lactic acid accumulation.

These two experiments with bicarbonate are perhaps complicated by the fact that the subject was somewhat nauseated when he began running.

TABLE 2

DATE	PREVIOUS TREATMENT	MAXIMUM L. A. CONCENTRATION	O_2 DEBT CALCULATED AFTER A. V. HILL	TIME OF PERFORMANCE	SPEED CONSTANT OF DISAPPEARANCE OF L. A.
		mgm. per 100 cc.	*liters*	*minutes*	
Oct. 14	—	121	12.70	4	0.0185
Oct. 21	20 g. $NaHCO_3$	136	11.26	$5\frac{1}{2}$	0.0186
Oct. 28	—	110	11.46	$5\frac{3}{4}$	0.020
Nov. 23	20 g. $NaHCO_3$	120	11.44	$6\frac{1}{2}$	0.023

The subject was left to decide when he had reached exhaustion and this decision may have been influenced by extraneous circumstances.

Effect of the oxygen tension on the amount of oxygen debt and on the duration of the performance. Averages of two experiments performed breathing a 40 per cent oxygen mixture and of two experiments breathing in air are compared in table 3 with one experiment performed breathing a 14 per cent O_2 mixture.

An increase in the percentage of oxygen in the inspired air seems to go parallel with a decrease in the capacity to accumulate lactic acid. It may be that a limit to the production of lactic acid is given by the acidity of the tissues. Working at a maximum metabolic rate, the CO_2 tension in the tissues is greater when a high oxygen percentage mixture is breathed, because the CO_2 production is greater, while the pulmonary ventilation, being at its maximum value, cannot vary whatever the oxygen content in the inspired air. Then if the tissue acidity limits lactic acid production, the capacity for accumulating lactic acid decreases with increasing amount

of oxygen in inspired air. This hypothesis is in agreement with the findings obtained in the bicarbonate experiments, which may be explained on the same lines.

On the assumption that the lactacid oxygen debt is proportional to the lactic acid content of the body at the end of work plus that produced during recovery, we conclude that also the lactacid oxygen debt is less when breathing high oxygen mixtures.

The speed constant of the lactacid process increases in the same direction as the amount of oxygen in the inspired air, which is in agreement with the hypothesis that the lactic acid removal is due to an oxidative process. Moreover, if the resynthesis of lactic acid to glycogen were due to the combustion of ordinary fuel, whose speed of oxidation is certainly very high, and if the slowness of removing lactic acid were due to the slowness

TABLE 3

DATE	MIXTURE BREATHED	TIME OF PERFORMANCE	MAXIMUM LACTIC ACID CONCENTRATION	INITIAL EXCESS LACTIC ACID CONCENTRATION AS CALCULATED (10^4)	OXYGEN INTAKE AT END OF EXERCISE	CALCULATED ALACTACID O_2 DEBT	CALCULATED LACTACID O_2 DEBT	SPEED CONSTANT OF DISAPPEARANCE OF LACTIC ACID	SPEED CONSTANT OF ALACTACID PROCESS
		minutes	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*liters per minute*	*liters*	*liters*		
Oct. 14	Air	4	115	150	3.85	2.5	6.16	0.0195	0.60
Oct. 28	Air	$5\frac{2}{3}$							
Nov. 11	O_2 40.5%	$7\frac{1}{3}$	84	108	4.5	2.5	4.43	0.027	0.60–0.65
Dec. 2	O_2 43.3%	$7\frac{2}{3}$	116	172	3.1	2.5	6.56	0.017	0.42
Jan. 23	O_2 13.64%	$2\frac{2}{3}$							

of the reaction lactic acid → glycogen, the speed of removing lactic acid would be unrelated to variation in oxygen tension. This finding supports the hypothesis that the slowness of the removal of lactic acid is due to the slowness of the oxidative process of combustion of the lactic acid itself.

We saw that for a constant content of oxygen in the inspired air the amount of alactacid oxygen debt is nearly proportional to the increase in rate of oxygen intake during exercise. It was interesting to see whether the amount of alactacid oxygen debt depended on one or the other of these two processes which run parallel. This problem can be solved by performing work at the same rate but at different rates of oxygen intake. This can be attained by breathing mixtures of various oxygen contents. From formula (6) the amount of alactacid oxygen debt is proportional to the extra oxygen consumption per minute in exercise over the resting and the lactacid oxygen consumption, and inversely proportional to the speed constant of

the alactacid mechanism. If this speed constant increases with an increase in work, the amount of alactacid oxygen debt remains constant. This is what really seems to happen: breathing a 13.6 per cent oxygen mixture the value of the speed constant for this process decreases to about 0.42 with a total alactacid oxygen debt of 2.5 liters. Breathing a 40 per cent oxygen mixture, though there seems to be an increase in the speed constant of the alactacid process, the increase in work metabolism is rather too small to affect this constant significantly.

It seems, therefore, that the amount of alactacid oxygen debt depends on the rate at which the work is carried on; this hypothesis, on the assumption that this oxygen debt is due to the resynthesis of the phosphagen, is in agreement with the current views on the part played by phosphagen in muscular contraction.

The values of the speed constants both for the alactacid and for the lactacid process decrease in the same direction as the oxygen content in the inspired air. Then we must expect that also the speed of payment of the total oxygen debt decreases with decreasing partial pressure of oxygen in the inspired air. This is in agreement with Margaria's (1928) findings on the speed of payment of the oxygen debt at low barometric pressure.

SUMMARY

1. The removal of lactic acid from the blood during recovery is, disregarding the first period, an exponential function of time: i.e., its speed of disappearance is proportional to the concentration of the lactic acid at that moment.

2. Evidence is given for the validity of the assumption that lactic acid is rapidly diffusible and uniformly distributed through the body, so that the concentration of lactic acid in the blood is proportional to the amount of lactic acid in the body at that time.

3. No extra lactic acid appears in the blood up to a rate of work corresponding to about $\frac{2}{3}$ of the maximum metabolic rate, after which the lactic acid increases very rapidly, with an increment of 7.0 grams per increment of 1 liter of O_2 debt as calculated after A. V. Hill.

4. The removal of lactic acid in the body is a very slow process, its velocity constant being 0.02; i.e., one half is removed in 15 minutes. Arguments are presented for the validity of the hypothesis that such a speed is limited by the slowness of the process of the oxidation of a fraction of the lactic acid itself.

5. After (3) and (4) it follows that the lactic acid mechanism would not play any important part in muscular contraction except in very strenuous exercise, probably in connection with the anaerobic conditions in which the exercise is performed.

6. The oxygen consumption curve during recovery may be considered in the simplest way as resulting from the sum of four functions:

a. The basal oxygen consumption as measured before the performance of work, a function independent of time.

b. An oxygen consumption attributable to oxidation of the lactic acid: this, as a function of time, is of an exponential character. This process is a very slow one, the value of the velocity constant being 0.02.

c. Another exponential function of time, occurring at a much faster rate, the velocity constant being 0.6 (50 per cent of the reaction takes place in $\frac{1}{2}$ minute).

d. An oxygen consumption decreasing during recovery very slowly so that this process may be present several hours after the end of the work; this function is not defined mathematically.

Of these four functions only *b* and *c* have the meaning of an oxygen debt payment. The function *d* has been interpreted as an increase of the resting metabolism caused by the exercise.

7. The mechanism of *b* is the lactic acid mechanism, as described by A. V. Hill. The mechanism *c* is independent of any lactic acid formation or removal, and therefore it is indicated in this paper as "alactacid."

8. The facts fit better in the four functions in (6) if we assume as coefficient of combustion of lactic acid 1:10 instead of 1:5. The value 1:10 seems therefore more probable, and this is supported also by a more reasonable value obtainable for the efficiency of the resynthesis of glycogen from lactic acid on such an assumption.

9. The alactacid oxygen debt is approximately a linear function of the oxygen intake in exercise. It is supposed to be related to the oxidation of substances (ordinary fuel) furnishing the energy for the resynthesis of phosphagen split down during muscular contraction. A rough calculation made on this assumption shows that that may be a possible interpretation. The maximum amount of oxygen debt by this mechanism was in our subject about 2.5 liters.

10. The lactacid oxygen debt starts coming into play only when there may be reasons to believe that the work is carried on in anaerobic condition. Its amount, relatively to the total amount of oxygen debt, increases particularly rapidly at the maximum rates of work. The maximum absolute amount is about 5 liters. It may be increased by increasing the capacity of the body to accumulate lactic acid, as for example, after ingestion of alkali.

11. The speed of payment of the alactacid oxygen debt and the speed of disappearance of lactic acid from the blood vary with the oxygen tension in the inspired air. Therefore, the payment of the entire oxygen debt is slower at low oxygen tensions.

12. The disappearance of lactic acid from the blood at the beginning of recovery after strenuous exercise shows a lag which does not seem to be fully explained either by a lag in the diffusion of lactic acid from muscles to the blood or by a slower oxidation of lactic acid, or by a delayed lactic acid production.

In a trained subject this lag has a duration of 6 or 8 minutes. In non-athletic and untrained subjects, incapable of good performances, the lag may last two or three times as long.

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LACTATION IN DIABETES

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In the course of experiments dealing with the effects of pregnancy upon the metabolism of diabetes, Markowitz and Soskin (1) found that pregnancy in 3 depancreatized bitches was not attended by growth of the mammary glands, nor by secretion of milk following parturition, although Markowitz and Simpson (2) had previously reported lactation in a single completely depancreatized animal, which suckled 2 pups for a period of one month. The absence of mammary hypertrophy and of secretory activity has also been observed by us in a bitch that became pregnant several months following total removal of the pancreas (3). The cause of this disturbance in the mechanism of lactation is not known. It may be tentatively assumed, however, that it is not related to the antidiabetic hormone, for the animals were maintained in good nutritional condition by daily injections of insulin.

The study of lactation in the diabetic animal involves considerable experimental difficulty, for although estrus has been regularly observed in a few of the completely depancreatized bitches maintained by one of us, the induction of pregnancy, with few exceptions, has not been successful In 2 completely depancreatized dogs, which were observed for a period of 4 years in Macleod's laboratory (4), pregnancy was produced in but one of these, and, although estrus occurred on subsequent occasions in the same animal, she never again became pregnant after mating. Moreover, when pancreatectomy is performed during the early part of pregnancy, absorption of the fetus or abortions are not uncommon sequelae.

The recently developed lactation hormone obtained from the anterior pituitary gland has greatly simplified the experimental approach to lactation, for not only does it eliminate the complications associated with pregnancy but it also evokes the secretion of milk in a relatively short space of time, which in the case of normal dogs may be as short as 3 days (5). This hormone is being used for further inquiries into the question of

[1] The expense of this investigation was defrayed in part by a grant to one of us (I. L. C.) from the Research Board of the University of California, Berkeley. The insulin was generously donated by the Eli Lilly Company.

lactation in the diabetic state. The present report deals with the effect of this hormone upon completely depancreatized dogs kept alive by daily injections of insulin.

METHODS AND MATERIALS. Following pancreatectomy the wounds were allowed to heal completely and the animals brought into good nutritional condition before the beginning of injections of the lactation hormone. This required at least 3 weeks. All depancreatized animals, with the exception of BR1, received twice daily, at 8:00 a.m. and at 4:00 p.m., a diet consisting of 200 grams of lean meat, 40 grams of sucrose, 5 grams of bone ash, and 5 cc. of cod liver oil. Vitamin B concentrate (6) was added to the diet at least 3 times per week. The animals were injected with 8 units of insulin at each time of feeding. The 24 hour sample of urine of the animals always contained glucose, in varying amounts from day to day.

The lactation-stimulating powders used in these experiments represent a fraction of an acid-acetone extract of dehydrated and defatted bovine anterior-pituitary powder. Complete details of its method of preparation will be given elsewhere (7). No assay of the powder employed in this investigation was attempted, the same powder being administered to depancreatized dogs and to normal controls.

RESULTS. Four normal dogs were used as controls in this study, and lactation was obtained in all. The amount of the hormone necessary to evoke the secretion of milk in these normal animals varied from 12 to 80 mgm., and the time of onset of lactation following the first injection varied from 2 to 6 days.

The results in the diabetic animals were not uniform, but, taken as a whole, they indicate that the lactation hormone may have no effect upon the completely depancreatized animal maintained with insulin. Seven experiments were performed on 6 completely depancreatized dogs. In 5 of these animals no secretion of milk was observed, despite the facts that the period of injection was prolonged in some cases to 17 days and that the amount of the hormone injected was increased in some cases to about 15 times the amount that was found necessary to induce lactation in the normal animals receiving the same hormone at the same time. Lactation occurred in a single depancreatized dog after 5 days of injection of the hormone. Both in respect to the time of onset of lactation and the amount of milk secreted, this animal did not differ markedly from the normal.

SERIES I. *SB-Control-3.* This animal was a normal adult bitch with resting mammaries. Its weight was 9.0 kgm. From June 4 to June 10, 1933 this animal received daily subcutaneous injections of 2 mgm. of lactation hormone L-14-2, and responded with a copious secretion of milk after the sixth injection.

Bri-Diabetic-10. This dog was completely depancreatized on May 1,

1932, and weighed 9.0 kgm. at the time of the experiment. The diet of this animal was similar to that recorded above, with the exception that the unsaponifiable portion of cod liver oil was supplied in the diet 3 times per week instead of raw cod liver oil. The animal was mature and in the di-estrum with resting mammaries. From June 3 to June 19, 1933 this animal was injected daily subcutaneously with 4 mgm. of the lactation hormone L-14-2. Despite 17 injections in which the animal received a total of 68 mgm. of the lactation principle, secretion of milk was not obtained from the mammary glands.

Wh-Diabetic-8. This adult animal was completely depancreatized on April 25, 1933 and weighed 9.2 kgm. at the time of the experiment. From June 3, 1933 to June 19, 1933 this animal received 17 subcutaneous injections of 4 mgm. each of the lactation factor L-14-2. No lactation occurred. At necropsy no pancreatic remnants were found after careful search; the mammary glands appeared normal.

SERIES II. *BL-Control-4.* This was a normal mature bitch weighing 8.5 kilograms. She lactated on July 13, 1933 after 2 subcutaneous injections of 20 mgm. each of lactation hormone L-17-2. After 2 subsequent injections of equal amounts of the hormone, this dog developed swollen milk lines and approximately 100 cc. of milk were obtained daily on 3 consecutive days. During the period of injection the urine was found to reduce Benedict's qualitative reagent.

R-Control-12. This was a normal adult bitch weighing 10.0 kgm. Its treatment and reaction throughout were identical with BL-Control-4.

Brn-Control-7. This normal adult bitch, weighing 6.0 kgm., received the same treatment as BL-Control-4. Four daily subcutaneous injections from July 13 to July 16, 1933 were necessary before lactation commenced. Reducing substances were present in the urine of this animal during the course of the experiment.

HIW-Diabetic-5. This was a mature bitch, weighing 6.5 kgm., in which lactation was induced on April 17, 1933 following the injections of a total of 40 mgm. of L-9-4 in 2 daily equal amounts. Bilateral ovariectomy had been performed on April 15, 1933 and complete pancreatectomy on May 23, 1933. From July 12 to July 22 this animal received daily injections of L-17-2, the same powder used in controls BL-4, R-12, and Brn-7. During this period, in which the animal was in excellent nutritional condition, she received in all 310 mgm. of the hormone. No effect was noticeable upon the mammary glands, which had not yet atrophied as the result of the ovariectomy, but which still appeared as normal resting glands.

FR-Diabetic-6. This animal was ovariectomized on April 10, 1933. Lactation was observed in this animal on April 17, 1933, following 6 daily injections of 20 mgm. of the lactation hormone (L-9-4). Its weight during

the course of lactation was 6.8 kgm. On May 18, 1933 this animal was completely depancreatized. From July 12 to July 22, 1933 she received 310 mgm. of prolactin (L-17-2). No lactation was obtained.

GNT-Diabetic-9. This animal was depancreatized on May 24, 1933. It received 310 mgm. of the lactation hormone (L-17-2) during a period of 11 days from July 11 to 22, 1933. No lactation was produced. During the period of injection the animal weighed 8.0 kgm.

Bri-Diabetic-10. This animal had already been used in series I on June 10, 1933. She came into estrum on July 5, 1933, although she had been completely depancreatized on May 1, 1932 and maintained on insulin for approximately 15 months. Since the mammaries in this bitch enlarged during this heat period, it was thought that this depancreatized dog, if any, might respond to the lactation factor. However, 210 mgm. of the potent L-17-2 given over a period of 10 days produced no noticeable effect on the mammary glands.

BNT-Diabetic-11. This bitch was depancreatized on May 22, 1933 and weighed 7.9 kgm. She lactated on July 16, 1933 after receiving 100 mgm. of L-17-2 in 5 daily subcutaneous injections of 20 mgm. each. Curiously enough, this dog had the smallest mammaries of the 6 bitches in the diabetic series, lactation being produced from poorly developed glands. At necropsy no remnants of the pancreas were found after careful search.

Discussion. The failure to demonstrate lactation in most of the completely depancreatized dogs after repeated injections of the hormone clearly shows that these animals, although maintained in apparently good nutritional condition with insulin, may not be normal in all respects. This, of course, has been suspected, inasmuch as the removal of the pancreas deprives the animal not only of the insulin-producing tissue, but of the zymogenous tissue as well. In the present investigation raw pancreas was not added to the diet of these animals, but this can be of no significance insofar as lactation is concerned, for in 3 depancreatized dogs receiving raw pancreas no lactation following pregnancy was observed by Markowitz and Soskin (1), whereas in the present study, in a single depancreatized dog receiving no raw pancreas, lactation was obtained with the hormone. In this respect it is of interest to note that the diabetic patient, in whom part of the pancreas is still functioning, may, in some cases at any rate, experience difficulty with lactation (8).

Although in the light of our present knowledge it is impossible to determine the nature of the disturbance so as to account for the inability of most of the depancreatized dogs under insulin treatment to secrete milk, it is of value nevertheless to consider the factors which may or may not be involved. The possibility that insulin directly inhibits milk secretion can be readily dismissed, since this hormone is present in the tissues of the normal animal. Although it was shown by Gowen and Tobey (9) that

the secretion of milk is reduced in cows by the injections of massive doses of insulin, this effect cannot be ascribed to the insulin *per se* but to the hypoglycemia that followed the injections of the hormone. The supposition of the presence of another pancreatic hormone having to do specifically with lactation seems to have no support in its favor, in view of the fact that in the single depancreatized dog in which lactation was observed, complete absence of pancreatic tissue was confirmed at necropsy. It is suggested that further studies on the altered metabolism of these animals may offer an explanation for the inhibition of lactation.

SUMMARY

Six completely depancreatized dogs that were kept alive by means of insulin and a special diet, and four normal dogs were injected with the lactation hormone obtained from the anterior pituitary gland. The secretion of milk was observed in all normal dogs. Five of the diabetic animals failed to lactate despite the fact that the amount of hormone injected was greater, and the period of its injection much longer than that found necessary to induce lactation in the normal dogs. In a single depancreatized dog lactation followed the administration of the hormone.

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SOME CONDITIONS AFFECTING INTENSITY AND DURATION THRESHOLDS IN MOTOR NERVE, WITH REFERENCE TO CHRONAXIE OF SUBORDINATION

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In 1929 Lapicque proposed that the selection of central nervous pathways during reflex coördination may be determined not by anatomical arrangements but rather by the relation of the chronaxies of adjacent neurones. To account for the modifiability of central coördination on this basis it is necessary to assume that the chronaxie of a given tissue is not stable. Accordingly, while retaining a "constitutional" chronaxie, which is fundamental to the neurone in question, Lapicque postulates in addition a "*chronaxie de subordination*," which may be observed when the neurone is under the influence of some other part of the nervous system. Two cases of this influence are possible: one in which the chronaxie of an efferent path is modified by a center, and another in which the center is itself modified, either by afferent stimulation or by local conditions. The theory is offered in explanation of the usual phenomena of reflex activity, including reflex reversal (switching) and inhibition.

The theory is based primarily upon experiments by M. Lapicque (1923) in which it was found that the chronaxie of the sciatic nerve of an intact frog was increased approximately 100 per cent when the nerve was severed in the lumbar region, or when the central nervous system was sectioned below the optic lobes. It was accordingly assumed that the center imposing the subordination was above this plane. Other evidence in support of the theory was found in the experiments of M. Lapicque (1923) and L. and M. Lapicque (1928a) upon the effect of anesthetics and of L. and M. Lapicque (1924 and 1928b) upon changes in chronaxie correlated with changes in afferent stimulation from the skin and muscles.

It seemed possible that the effect observed in these fundamental experiments might have been due to the operation itself rather than to the exclusion of a higher center. In order to control specifically for the effects of injury, Dr. H. Davis suggested to us the use of a reversible cold block which had previously been found practical in the study of central reflex phenomena (Beaman and Davis, 1931), and which should cut off the subordinating influence from any part peripheral to it without introducing

any very significant stimulation. In an experiment upon the spinal cord and peroneal nerve of a decerebrate cat, a spinal block was not established that could be shown to be complete. In the peroneal nerve itself a complete block was obtained as shown by absence of response in M. tibialis to maximal motor stimulation applied central to the block. In this experiment the chronaxie of the nerve stimulated below the block was unchanged. Some part of the higher control may be lost in the decerebrate preparation, but, as we shall note later, the afferent and mesencephalic influence upon the motor neurone in decerebrate rigidity calls for a significant degree of subordination, unless Lapicque's theory is to be severely limited in its application. After obtaining this negative result we cut the nerve 3 cm. from the electrodes and again obtained no change. A cut 2 cm. away also gave no change. But a cut less than 1 cm. from the electrodes gave a significant increase in chronaxie—for a short time as great as 60 per cent, dropping finally to about 15 per cent. These changes were definitely correlated with important changes in the rheobase. This pointed clearly to an effect of injury near the region of stimulation. The cold block was, then, dispensed with as a somewhat doubtful method, and the distance between the electrodes and the cut was used instead as the controlled variable.

The experiments reported here are concerned with the influence of the distance of the cut from the point of stimulation upon the rheobase and chronaxie, and with the nature of the resulting change as shown in the total intensity-duration curve of the current required for excitation. It also seemed important to re-examine other experimental evidence which led to the concept of chronaxie of subordination. Experiments of this character are also included in the present report.

METHOD. In all cases the duration of the stimulus was determined by the charge of a condenser. The current for charging the condenser was drawn from a potentiometer supplied by a series of dry cells of which the number could be varied at will. To double the rheobasic intensity for chronaxie measurements, a fixed resistance equal to the total resistance of the potentiometer was cut out by a double throw switch which also put into the circuit, in place of the fixed 0.25 μf condenser, the variable decade condenser which had a range of capacities from 1 to 0.001 microfarad. Gradations to 0.0001 microfarad were obtained when necessary by an air condenser placed in parallel.

In a few experiments voltages two, three and four times the rheobase were obtained by reducing proportionately the total resistance in the potentiometer circuit. In all the later experiments fixed voltage increments were obtained by adding one-half, two, three or four cells in series in the condenser circuit (cf. fig. 1). In this way the rheobase potentiometer setting could be left undisturbed and absolute increments above that value

obtained by manipulation of a single selector switch. The advantage of this method lay in the speed with which an extensive intensity-duration curve could be obtained and in the possibility of direct comparison of data, the occasion for which will be explained below.

The experiments were performed on 13 cats and 10 frogs. The cats were decerebrated by the trephine method under deep ether anesthesia; the peroneal or popliteal nerves were prepared for stimulation, and the cats allowed to recover completely from shock and anesthesia before measurements were recorded. The frogs were treated similarly, being either decorticated or decerebrated under ether anesthesia. In two cases anesthetized intact frogs were allowed to recover until spontaneous movements appeared, and immediately anesthetized again for comparative observations.

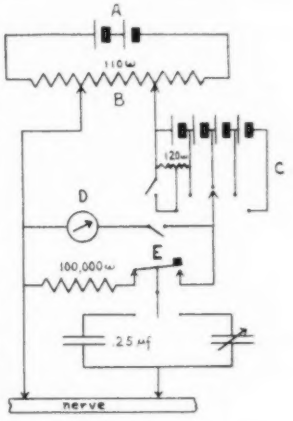

Fig. 1. Simplified diagram of apparatus used for recording I-T curves by the voltage increment method. A = source of potential for rheobase; B = potentiometer; C = series of dry cells and selector switch used for adding voltage increments; D = voltmeter; E = telegraph key for charge and discharge of condensers.

Throughout the experiments tube electrodes of the type described by Forbes, Davis and Lambert (1930) were used for stimulation. The silver wires of approximately 25 B and S gauge were placed from 5 to 9 mm. apart in insulating material of size and weight adapted to the particular needs of the preparation. Since care was taken that extra moisture should not accumulate in the tubes, the possibility of the introduction of an "α effect" should have been small (cf. L. Lapicque, 1931b). The results obtained upon cutting the nerve could certainly not be explained by such a phenomenon.

The threshold response of the nerve as indicated by contraction of the muscle was observed by a second experimenter who was unaware of the settings of condenser or resistance box. The method employed for detecting the least contraction varied with the preparation; in the cat, the least movement of the hairs directly above the muscle, or the pull of the tendon upon a lever served as indicator, while in the frog the twitch of the muscle was observed through the skin. Usually the end point was clearly reproducible. As a rule several determinations of chronaxie or the total I-T curve were made before cutting the nerve. It has been objected that because of the extreme variability of the chronaxie during subordination it would be impossible to obtain a complete I-T curve. Complete and consistently reproducible curves and chronaxies were nevertheless

obtained under conditions which we consider adequate for revealing a subordinating influence of higher centers (cf. discussion). In most cases determinations were taken as soon after cutting the nerve as possible, in order to note the maximum effect of the injury upon the rheobase and chronaxie or total I-T curve. The fairly rapid return of the rheobase toward its initial value, even during the first minute after an effective section, introduced a slight discrepancy between the curve as determined and that which would have been found if the voltage increment had been progressively adjusted to the changing rheobase. The amount of this discrepancy was never large. Observations were made for several minutes afterward to follow whatever further recovery there might be. Special care was taken not to disturb the position of the nerve upon the electrodes during the cutting, in order to avoid errors due to changes of contact with the same fibres or an actual change in the group of fibres stimulated.

RESULTS. *A. Effect of section of the peripheral nerve.* Our preliminary experiments indicated that a change in chronaxie correlated with a change in rheobase was obtained upon cutting the nerve close to the electrodes. If the nerve was severed at a great enough distance from the point of stimulation, little or no change in either chronaxie or rheobase was observed.

In order to determine in what way this change in excitation time would appear in a more extensive I-T curve, four experiments upon cats were performed in which durations were measured at voltages of two, three, and, in most of the cases, four times the rheobase. In two of these a slight decrease in chronaxie correlated with a slight increase in rheobase was observed when the nerve was cut approximately 4 cm. central to the electrodes. In the other two cases there was no appreciable change. As the nerve was sectioned nearer and nearer the electrodes, there was a progressive decrease in the rheobase until, with a cut made at approximately 1 cm. from the point of stimulation, there was in every case a marked drop in rheobase and a corresponding increase in durations at the different intensities. The reduction in rheobase averaged 0.36 volt or about 35 per cent of the original average value. The average increase in chronaxie was 50 per cent, while at three and four times the rheobase the increases in durations were 41 per cent and 33 per cent respectively. Thus, although the increased durations at each point would indicate an apparent increase in excitation time, the progressive decrease in percentage change, as the voltage increased, shows that there was not a simple increase in the time characteristic as a measure of the change by chronaxie would imply, but a change of a different sort.

Furthermore, when the data for these experiments are plotted as intensity-duration curves, it is apparent that the principal change due to the last cut is a downward shift of the curve. This is shown in figure 2, which represents a typical experiment. The original curve is the upper line;

upon cutting the nerve near the electrodes the curve shifts to the lower position.

Figure 2 also illustrates the following important fact. If the curves obtained before and after cutting are plotted so that the ordinates represent *absolute voltage increments above the rheobase* in each case, they are very nearly superimposed. Complete superimposition would be expected if

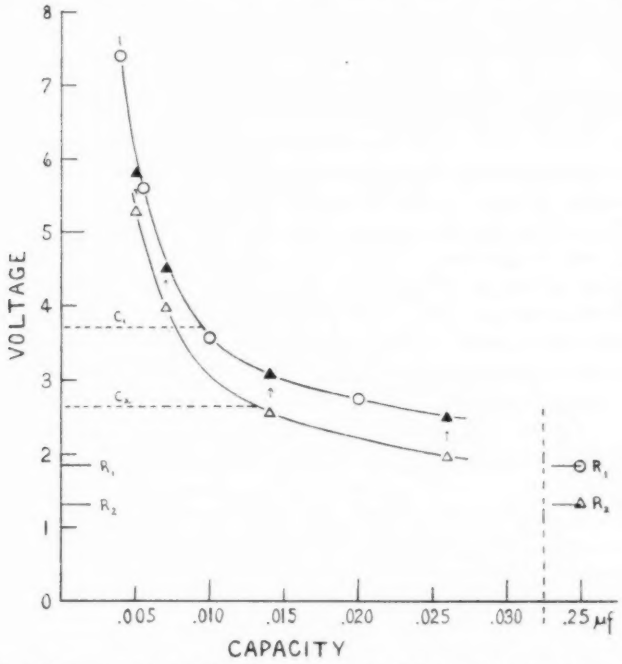

Fig. 2. I-T curves taken with voltages 1½, 2, 3 and 4 times the rheobase. Preparation: peroneal nerve of a decerebrate cat. Open circles, curve before cutting; open triangles, curve 1 minute after cutting 0.6 cm. from the anode; solid triangles, lower curve shifted vertically a distance equal to change in rheobase, i.e., plotted with $R = 0$ and the ordinate representing absolute voltages above the rheobase. Change in time parameter, 0. Increase in chronaxie, 0.04 μf.

the only change occurring was in a parameter such as a of the Weiss formula $i = a + \frac{b}{t}$. That the principal change resulting from injury is largely of this sort, simply a change in base-line for the whole curve, was shown in a test by the Weiss formula of the curves obtained in a typical experiment. These gave a good approximate fit in the region observed, which is to be expected since neither extremely long nor short durations

were involved. Thus in figure 2 the solid triangles represent the position of the lower curve shifted vertically a distance equal to just the change in rheobase observed upon cutting. In all four of these experiments, if we make this allowance for a change in rheobase, there is practically no change in the curve. The cut affects principally the rheobase and has little effect upon the time factor.[1] This led to the hypothesis, discussed below, that cutting the nerve near the point of stimulation simply added a constant excitatory effect to the stimulating current. We therefore devised the method already described for measuring durations at fixed voltage increments above the rheobase (cf. method). The validity of this method is shown by plotting the data so obtained. This gives essentially $V-R$ as a function of T. These curves are more nearly superimposable than are those representing V/R as a function of T. Changes in the time factor independent of changes in rheobase could thus be directly observed.

The effect of cutting the nerve at various distances from the stimulating electrodes was recorded by this method in 22 cases. Half of these were entire sciatic nerves of spinal frogs, the twitch of the muscle having the lowest threshold being used as the indicator. Of the 11 experiments on decerebrate cats, 6 were on peroneal nerves and 5 on popliteal. Since the changes ensuing upon cutting are found to be similar in both cat and frog, they are treated together in the following discussion. For comparison the curves before and after cutting were plotted logarithmically with rheobases superimposed to show as clearly as possible any change in the time factor.

In 11 cases (7 cat and 4 frog), the first cuts were made at distances of 1.2 to 4 cm. from the anode. The change in rheobase in this group averaged ± 11 per cent. This value is slightly larger than the total average value, 8.6 per cent for 20 cases in which cuts were made outside the effective range of the injury. These include the four cases previously mentioned, 6 others which are not otherwise noted since the duration curves were imperfectly recorded, and the 11 cases of the group considered here. In 7 of the latter (5 cat and 2 frog) there was no change in the intensity-duration curve from that of the intact nerve. In the other four cases there were measurable but inconsistent changes in the time factor; two showed an increase and two a decrease, but without regular correlation with changes in rheobase or absolute distance from the electrodes. The chronaxies derived from the curves were in 6 of these 11 cases unchanged; of the others, all but one changed less than 10 per cent. In that one, the cut was made less than 3 cm. from the anode and the effect may have been due to the in-

[1] "Time parameter" and "time factor" are used interchangeably throughout the text. Parameter is, of course, the more accurate term, mathematically speaking. In this case, the time parameter referred to is b in the Weiss formula noted above.

fluence of the injury since its effective range varied markedly in different preparations and under different experimental conditions.

Further cuts were then made until the effect upon the rheobase increased to a maximum. The curves chosen for comparison are with a few exceptions those for which there was the greatest total change in rheobase from that of the uncut nerve. The most effective cut was in general within 1 cm. of the anode (descending shocks were used throughout this series, although the effect of injury was found to be equally pronounced with ascending shocks). In the other 11 cases one cut only was made, presumably within the range of influence of the injury. The results of all cuts made near the electrodes were essentially the same and are here considered together.

The most noticeable effect upon cutting the nerve near the electrodes was the large drop in rheobasic voltage, an average change of 40 per cent from the initial value. There was a single exceptional case, in which there was an increase in rheobase. In 15 of the 22 cases there was a shift of the I-T curve to the left along the time axis, in 2 cases no change at all from that of the uncut nerve and in 5 cases a shift to the right. One of the latter was associated with the increase in rheobase. For the others no consistent correlation could be shown between the amount of change of the time factor in either direction, and the percentage or absolute drop in rheobase.

If a typical set of curves be considered, such as those in figure 3B, it is seen that, after compensation for the change in the voltage parameter is made by superimposing the rheobases, there is a distinct although slight shift of the curve to the left, i.e., the injury has also had an effect on the time parameter. If the change in time is then compensated for by shifting the curve to the right, it will be seen that the curve (dotted line and triangles) still does not completely superimpose and that there is a slight but perceptible difference in its shape. This change was, in 14 of the 15 cases which shift to the left, toward a greater concavity, whereas, in all of the cases which shift to the right, it was toward a decreased concavity from that of the uncut nerve. This indicates the probable influence of the injury upon a third parameter, as well as upon the time and voltage parameters; hence the necessity of invoking a third parameter (cf. Bouckaert, 1932; Rosenblueth and Rioch, 1933) to express the excitability of the nerve under these conditions.

Figure 3A and B show all of these changes clearly. In figure 3A the first cut made at 3.75 cm. from the anode caused an insignificant drop in rheobase of 0.02 volt. The subsequent curve indicated by crosses shows no change in the time factor or chronaxie. The curve plotted with triangles (solid line, 3B) shows the characteristic change due to the effect of injury, a marked drop in rheobase. If the rheobases are superimposed (broken

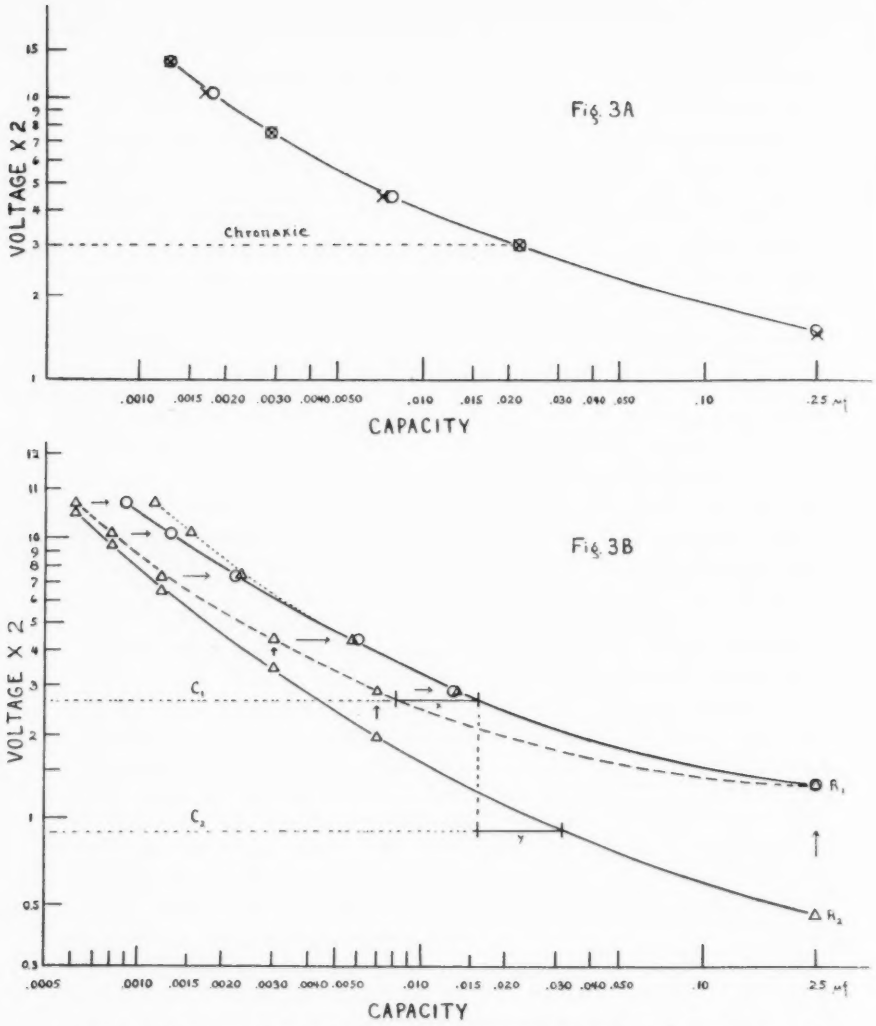

Fig. 3. I-T curves taken by voltage increment method and plotted on a logarithmic scale.

A. Effect of a cut 3.75 cm. from the anode. Frog sciatic nerve. Circles, curve before cutting; crosses, curve after cutting. Drop in rheobase, 0.02 V. Change in time parameter and chronaxie, 0.

B. Effect of cutting a peroneal nerve of a decerebrate cat 2.5 cm. above the anode. Circles, curve before cutting; triangles (solid line), after cutting; triangles (broken line), same, plotted with voltage increments equal to the drop in rheobase added to each point; triangles (dotted line), same with difference in time parameter added to each point. Shifts indicated by arrows. Note that with neither shift is there complete superimposition of the curves. Drop in rheobase, 0.43 V. Decrease in time parameter (x), 0.08 μf; increase in chronaxie (y), 0.021 μf.

line) a significant decrease in the time factor is seen (x). If, however, the chronaxie is taken as a measure, a marked increase is found (y).

In general then it may be said that a cut made beyond the effective range of the injury causes minor changes in the rheobasic voltage (less than 10 per cent in 15 out of 20 cases, and 20 per cent in only one of the other 5), and no change in the time factor from that of the nerve connected with the central nervous system. On the other hand, a cut made within the effective range of the injury causes 1, a marked decrease in the voltage parameter; 2, a smaller decrease in the time parameter, and 3, a slight change in a third parameter X. The most noticeable of these is the change in rheobase. The bearing of these observations upon chronaxie of subordination will be considered later.

B. Effect of sections in the central nervous system. Large changes in rheobase and chronaxie are described by M. Lapicque (1923) upon sectioning the brain stem below the optic lobes. Such an effect has been shown in the preceding section to be dependent upon the proximity of the cut to the electrodes. It seemed essential, therefore, to repeat these experiments, since a cut at this distance obviously should have no injury effect upon the region of the nerve stimulated.

A preliminary experiment on a decerebrate cat, in which the cord was cut at the level of the last rib, showed a decrease in rheobase of 7 per cent and no change in chronaxie, though later cutting of the nerve itself gave large increases in chronaxie and corresponding decreases in rheobase. In four other experiments upon frogs, curves were taken by the standard increment method, which were later analyzed by double logarithmic plots. Two of the frogs were decerebrated, whereas in the other two, the entire optic lobes were intact. Of the two decerebrate preparations one showed a 7 per cent decrease in rheobase upon pithing; the other, no change. In neither was there any change in the time factor. Of the other two, one showed a slight decrease in rheobase (8 per cent) when the optic lobes were removed and an increase in rheobase (12 per cent) when the frog was pithed. There was absolutely no observable change in the time factor. The fourth, in which the central nervous system was even more nearly intact, showed no change in the rheobase or time factor when the cord was cut below the medulla. A fifth frog with optic lobes intact showed no change in rheobase when the medulla was destroyed. No curves were taken in this case. In these cases the chronaxie, derived from the curve, never changed more than 16 per cent; when the rheobase remained constant, so did the chronaxie; the small changes in rheobase were correlated with small inverse changes in chronaxie. In all cases further cuts made on the nerves gave the characteristic results described above. Thus none of our results furnished consistent evidence of "subordination."

C. Effect of anesthesia. M. Lapicque (1923) also states that during

anesthetization by chloroform the chronaxie of the nerve becomes the same as that of the nerve cut away from the central nervous system, showing that chronaxie of subordination is not an effect of injury, but is definitely dependent upon synaptic conjunction of the central nervous system with the motor nerve. Variation of the chronaxie according to the depth of anesthesia has been noted in several of the Lapicque experiments, and the change from the stability of the chronaxie under deep anesthesia to great instability without anesthesia has been described as one of the chief characteristics of chronaxie of subordination (cf. L. and M. Lapicque, 1924). Changes in the ratio of the chronaxies of two different nerves during anesthesia have also been described (L. and M. Lapicque, 1928b).

In order to determine whether the variability of the chronaxie and its change under anesthesia might not be due to changes in rheobase independent of changes in the time factor, two experiments upon frogs were performed and one upon a decerebrate cat. In two, ether was used, in the other, chloroform.

In the experiments on frogs, curves were taken as soon as they had been thoroughly anesthetized. The anesthetic was then removed and curves were taken while the anesthetic wore off until spontaneous movements appeared. The anesthetic was again administered, and the procedure reversed. This was repeated several times. In the experiment on the cat the same procedure was followed except that before ether was given the animal was allowed to recover from shock following decerebration and to develop rigidity, the absence of which could serve as an indicator of synaptic disjunction during anesthetization.

In all three experiments there was no change in the time factor, as recorded by the voltage increment method, that could be correlated with depth of anesthesia. There was in one case a slight progressive decrease throughout the experiment; in the others no change at all. For the peroneal nerve of the cat there was a progressive increase correlated with an increase in fluid around the electrodes, whereas for the popliteal nerve, which should, according to Lapicque (1928b), show a marked degree of subordination, there was no change at all.

One of the frog preparations showed a marked progressive decrease in rheobase regardless of the state of anesthesia. In the other, there were slight variations in both directions, but uncorrelated with the depth of anesthesia. Both nerves of the cat gave a slight progressive increase in rheobase throughout the experiment. In none of the experiments were there changes in rheobase and chronaxie of the type and order of magnitude reported by M. Lapicque. It should be noted, however, that in one experiment when the frog was well out of anesthesia violent spasms pulled the leg away from the electrodes, so that the nerve was badly stretched.

Large variations in rheobase and some change in the time factor resulted. This suggests the possibility that the effects observed by the Lapicques may have been due to change of contact of the electrodes or stretching of the nerve during activity, since it has been stressed that very active specimens are necessary to demonstrate the presence of chronaxie of subordination (cf. Monnier and Jasper, 1932; L. and M. Lapicque, 1928b).

D. Effect of proprioceptive stimulation. L. and M. Lapicque (1924) ascribed to chronaxie of subordination a dependence upon afferent stimulation from the skin and consequently upon the degree of reflex tone, since, according to Pieron and Ozorio de Almeida (1924) muscular tone in the frog is largely dependent on afferent stimulation from the skin. In 1928b L. and M. Lapicque attributed the origin of chronaxie of subordination also to the proprioceptive stimuli from the muscle itself and gave as evidence experiments in which the foot of a frog "*vigoureuse et agile décérébrée depuis 4 à 5 heures*" was passively flexed and extended, giving a corresponding reduction and increase in chronaxie with the stretch and relaxation of the gastrocnemius muscle. They also found reduced chronaxies when the muscle was under tension produced by attaching weights directly to the tendon.

We repeated these experiments on 5 frogs with optic lobes intact and on 2 cats decerebrated at the usual level. Curves were taken by the voltage increment method. Tensions were increased in two experiments on frogs by hanging weights of 10 to 50 grams on the tendon in one case and by stretching the muscle up to 10, 32, 46 and 66 grams tension in the other case. There were no changes in the time factor, chronaxie or the rheobase.

In four of the six flexion-extension experiments there was no change in the time factor. In the other two, there were progressive changes only. All of the frogs showed reflex activity to various stimuli throughout the observations and the cats showed increased extensor thrust in response to flexing the foot, indicating active centers, and in the last case, increased proprioceptive impulses to the centers. In the experiments upon cats, electrodes were placed on both popliteal and peroneal nerve and curves were recorded by observation of the gastrocnemius and tibialis muscles. Neither showed a change in the time factor.

It is interesting to note, however, that there were changes in rheobase. These were in general less than 10 per cent and were in all but one case unrelated to changes in muscular tone or position. In that one case the correlated change was great enough to give an 11 percent change in chronaxie, though there was absolutely no change in the time factor as measured by the voltage increment method.

Discussion. In all of our experiments, although our animals were obviously reflexly active, the only observed changes in rheobase or chronaxie that were comparable to those said to result from release of the

peripheral nerve from central control were those definitely correlated with proximity of the section in the nerve to the electrodes. This indicated an effect of injury which might be due to the action of the injury current, for, according to Adrian (1930), such a current might, in the case of a motor nerve, partially depolarize the tissue and therefore act as a partial stimulus. The progressively lowered rheobase noted in section *A* with the approach of the cut to the electrodes would then be due largely to the fact that the nerve was already partially depolarized by the injury current and therefore required less additional energy to complete the depolarization necessary for excitation. Thus the base-line for the I-T curve would simply be shifted downward and the shape of the curve would be unchanged (cf. fig. 2). That the total quantity of depolarization necessary for excitation might also be slightly decreased is indicated by the shortening of the time factor (cf. section *A*). This, together, with the lowered rheobase, may be interpreted as an increased excitability of the nerve.

The significance of this change in rheobase as related to the I-T curve, the true nature of the change in the time parameter, as well as the change in the third parameter X noted in section *A*, are not revealed by measurements of chronaxie and rheobase alone. Therefore, chronaxie is an inadequate indicator of the intensity-duration relationship under conditions of injury. This is clearly illustrated in figures 2 and 3 (section *A*) which show that measurements by chronaxie of the changes in the time factor give an increase after injury, whereas measurements by the voltage increment method reveal a decrease. It might be objected that since, among the curves shown in section *A*, only those of the intact nerve approach the canonical curve described by Lapicque (1931a), the chronaxie of the nerve in the region of injury would be a "pseudo-chronaxie" and would, therefore, be erroneous as a measure of the time factor anyway. It is, however, very close to the value calculated from the Weiss formula, which yields a value for chronaxie inversely proportional to the rheobase. Whatever the duration taken at double the absolute rheobase signifies, it is in these cases an erroneous indicator of the time factor, for it shows an increase where there is actually a decrease. It should be noted that throughout the experiments in support of the theory of chronaxie of subordination there were apparently no checks as to whether the chronaxies obtained were "pseudo" or real chronaxies.

Of the recent experiments dealing with chronaxie of subordination including those by A. B. Chauchard and Paul Chauchard (1933), P. Denissoff (1933), H. H. Jasper (1932 and 1933) with the exception of those by A. M. Monnier and H. H. Jasper (1932a and b) who measured velocity of conduction and size of action current, the general criticism may be made that all the measurements of changes in excitability were made with chronaxie. Our own experiments reported above indicate the type of error into which

the use of chronaxie may lead in cases in which the rheobase is changing. We feel that this criticism is particularly pertinent because the injury mimics so completely the effects described by Lapicque and others for the release of the peripheral nerve from central control. In fact, Lapicque (1924) describes chronaxie of subordination as a functional polarization characterized by a diminution of chronaxie and elevation of the rheobase proportional to the influence of the higher centers, which is revealed by a decrease in rheobase and increase in chronaxie upon cutting the nerve away from the central nervous system.

A further parallelism between the effect of release from chronaxie of subordination and injury is to be found in the description by Adrian of the conditions for a maximum stimulating effect of the injury current such as a thick, intact sheath and a well irrigated nerve. These conditions coincide conspicuously with the optimal conditions for revealing the effects of chronaxie of subordination described by Monnier and Jasper (footnote, 1932a).

It must be pointed out, however, that in the experiments of M. Lapicque (1923) and in those of Monnier and Jasper (1932) and others supporting the theory the nerve was cut at a distance from the electrodes apparently great enough to eliminate the effect of injury. Large changes in rheobase and chronaxie nevertheless were reported. Our experiments indicate that changes in rheobase and chronaxie seldom exceed 10 per cent, either upon cutting the nerve outside the range of injury or upon cutting the spinal cord and brain stem. With these relatively small fluctuations in the rheobase there were absolutely no correlated changes in the time factor when measured by the voltage increment method.

A repetition of other experiments given in support of the theory, namely, those upon the effects of anesthesia, and the effects of proprioceptive stimulation resulting from flexion and extension of the foot, failed to show the correlated changes in rheobase found by the Lapicques (1923, 1928a, 1928b) or to show any changes in the time factor when measured by the voltage increment method.[2] We believe, therefore, that under the conditions of these experiments the central nervous system has no subordinating effect on the voltage or time factors; and that in general chronaxie of subordination may well be an artefact dependent upon fluctuations in rheobase which may be ascribed in the experiments on cutting the cord to a shift of electrodes (cf. sections *C* and *D*), or in those upon cutting the nerve to the effect of injury which varies in different preparations in the range of its effectiveness, according to different conditions of moisture, etc., and might include cuts made in the lumbar region of the nerve.

[2] Our attention has been called to the fact that according to the hypothesis set forth by Monnier and Jasper (1932b) no change in the time factor thus measured would be expected under subordination.

Since a change in rheobase comparable in magnitude to that caused by injury has been reported so many times as a result of release from subordination, it might be objected that the preparations used here were incapable of exhibiting the effect because of the absence of part of the mesencephalon in decerebrate preparations. In answer to this objection it may be said that all measurements were made on preparations that were reflexly active. If chronaxie of subordination is to explain coördination and choice of pathways, excitation and inhibition, as Lapicque (1929) claims, the effect must certainly be present in all preparations which exhibit these coördinating functions, whether they be spinal, decerebrate or intact. Not only has the effect been reported in decerebrate preparations by Lapicque himself (cf. L. and M. Lapicque, 1928b), but also, since L. and M. Lapicque (1928b) suggested a postural origin for chronaxie de subordination, a decerebrate preparation with the exaggerated postural tone typical of decerebrate rigidity should show a maximal amount of subordination.

An examination of the arguments for the theory and the experimental evidence on which it is based shows, however, certain inconsistencies.

1. Lapicque states (1929) that in the flexion reflex of a spinal preparation the selection by the afferent stimulus of the flexor motor neurone rather than the extensor motor neurone could be explained by the Bourguinon 2:1 ratio of the chronaxies of these antagonists, and refers to the equalizing effect of strychnine found by Bremer and Rylant (1924, 1925) as proof. In 1928a L. and M. Lapicque had already reported experiments in which it was found that cutting away the mesencephalon gave an equalization of the 2:1 ratio, thereby proving that the mesencephalon was the subordinating center controlling the chronaxies of these antagonists. Nevertheless, the flexion reflex is very evident in a spinal preparation.

2. It is maintained by Lapicque (1929) also, that inhibition could be explained by disjunction in a pathway resulting from the heterochronism of two adjacent neurones imposed upon them by a higher center. Inhibition certainly exists in the spinal preparation. Yet, by the primary experiment of M. Lapicque (1923) and others, the center controlling the chronaxies of peripheral neurones was located in the mesencephalon. If this were so, the possibility of having inhibition in the spinal preparation would be automatically removed.

3. Bonvallet and Rudeanu (1932), in the case of the dog, localized the center controlling the 2:1 ratio of the flexors and extensors in the mesencephalon, thereby corroborating the work of L. and M. Lapicque on the frog in 1928. Rudeanu and Bonvallet (1932) further noted that the equalization of chronaxies of the extensor and flexor groups found by them after removal of the mesencephalon seemed to be correlated with decerebrate rigidity. It is well known that decerebrate rigidity is characterized by augmentation of the myotatic or stretch reflex with increased excitation

of the extensors and inhibition of the flexors. This phenomenon could, then, hardly be explained by equalization of the chronaxies, for, on the basis of Lapicque's theory, a state of rigidity due to equalization would involve the possibility of both flexors and extensors being equally stimulated. In fact, since L. and M. Lapicque (1928b) had already ascribed a postural origin to chronaxie of subordination, a condition of decerebrate rigidity which has been described as an exaggerated postural reflex would seem to be ideal for showing a maximal amount of subordination. Thus the claim of Rudeanu and Bonvallet that in decerebrate rigidity the center for chronaxie of subordination has been removed can hardly be advanced in support of Lapicque's theory. It cannot be assumed that the rigidity of decerebration is comparable to that described for strychnine rigidity in which both extensors and flexors are equally stimulated or to the state of incoördination, after morphine, etc., ascribed by Rudeanu and Bonvallet (1931) to the equalization of the ratios of the chronaxies of extensors and flexors, for coördinated movements are not only evident during a condition of decerebrate rigidity but the state is itself a highly coördinated, although exaggerated, postural response.

It may be concluded, therefore, that chronaxie has been shown to be an inadequate measure of the time factor of excitation under conditions of changing rheobase which are closely similar to those obtaining in the experiments upon which the theory of chronaxie of subordination is based; that under conditions which should be adequate to reveal subordination by the central nervous system we could demonstrate only minor changes in rheobase and chronaxie and no change in the time factor of excitation as revealed by the voltage increment method; and, finally, that the theory of "chronaxic switching" as a basis of reflex coördination is inconsistent with the experimental evidence advanced for it and with other well established facts.

We are indebted to Drs. H. Davis, A. Rosenblueth and D. McK. Rioch for their valuable advice and criticism in the preparation of this paper.

SUMMARY

1. In experiments performed upon decerebrate cats and frogs, a significant decrease in rheobase and increase in chronaxie, such as that described by M. Lapicque (1923) upon the release of the peripheral nerve from central control, was found to be correlated only with the proximity of the section in the nerve to the electrodes (cf. section *A*).

2. *a*. There was almost complete superimposition of the curves representing durations taken at two, three and four times the rheobase when a fixed voltage increment equal to the drop in rheobase was added to each point.

b. This led to the hypothesis that the lowered rheobase or shift of baseline of the curve was due to a partial stimulation of the nerve by the injury current such as described by Adrian (1930).

c. Consequently a method was devised for taking duration measurements at fixed increments of voltage above the rheobase, for more direct comparison of changes in the time factor.

3. A careful analysis of data obtained by this method by double logarithmic plots showed that the principal changes occurring as a result of injury are *a*, a decrease in rheobase, *b*, a slight but definite decrease in the time parameter, rather than the increase indicated by chronaxic measurements, and *c*, a slight change in a third parameter X (cf. section *A*).

4. It was concluded that chronaxie is an inaccurate measure of changes in the time parameter under conditions of changing rheobase such as are reported to occur upon release of the peripheral nerve from subordination (cf. discussion).

5. The changes observed in rheobase and chronaxie when the nerve was sectioned outside the effective range of the injury were relatively insignificant, seldom exceeding 10 per cent. There was in most cases no change in the time factor as revealed by the voltage increment method (cf. section *A*).

6. Further experiments upon the effects of cutting the cord, of anesthesia and of proprioceptive stimulation failed to confirm the results reported by the Lapicques (cf. sections *B*, *C* and *D*). Thus the primary experiments in support of the theory of "*chronaxie de subordination*" were not corroborated.

7. An examination of the arguments advanced in support of the theory of "*chronaxie de subordination*" as a basis of central coördination, reveals several inconsistencies with the evidence advanced for it and with other well established facts (cf. discussion).

ADDENDUM

Doctor Jasper has kindly called our attention to the observations of M. Lapicque and F. Vahl (1931) and M. Lapicque (1932) in which "subordination" was found in a spinal frog with a small dose of caffeine or strychnine. This and more recently published evidence by H. H. Jasper (1933) has led them to abandon the view that the center for subordination is necessarily in the mesencephalon. Our criticism on that point, therefore, applies to a view which has been modified by the recent findings in Lapicque's laboratory.

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HYPERGUANIDINEMIA ASSOCIATED WITH DEHYDRATION IN NORMAL AND IN PARATHYROIDECTOMIZED DOGS

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It is generally agreed that the tetany which follows parathyroidectomy is primarily the result of a decrease in active calcium in the blood. However, the severity of the tremors and convulsions, the time of onset of tetany, and the level of serum calcium at which tetany occurs, show marked variation in different experimental subjects. Alkalosis is known to increase the severity of parathyroid tetany by depressing the ionization of calcium. In this connection it has been previously shown that the alkalosis which results, especially in hot weather, from a rise in temperature and consequent panting plays a large rôle in hastening the onset and increasing the severity of the attacks (1). There remain, however, certain instances of tetany occurring when the serum calcium is only moderately reduced and in the absence of alkalosis which suggest that some other factor is concerned.

Considerable attention has been given in the past to the theory that parathyroid tetany is caused by some toxic substance which accumulates following removal of the parathyroid glands. The theory which has received most attention is that of Paton (15) (16) who concluded that parathyroid tetany is due to guanidine intoxication. Further work which apparently supports this view is the observation that parathyroidectomized animals are more susceptible to guanidine intoxication than normal animals (5) and the confirmation by various investigators (3) (10) (20) (17) that guanidine is increased in the blood of parathyroidectomized dogs. Against this theory is the failure on the part of numerous investigators either to find an increase in guanidine in the blood during parathyroid tetany or to correlate the metabolism of guanidine bases with the function of the parathyroid glands (12) (18) (8) (7) (19).

In our recent study (2) of blood from 28 parathyroidectomized dogs an increase in guanidine was observed in only about 50 per cent of the subjects, thus definitely excluding hyperguanidinemia as the primary cause

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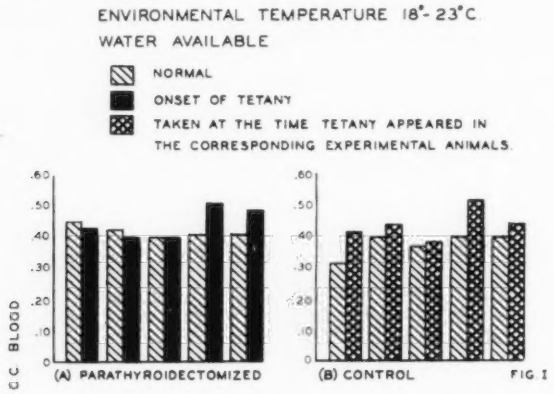
of tetany. The high guanidine values occurred without apparent uniformity of conditions except in one group of four dogs studied during the extremely hot weather of the summer months. There was a normal concentration of guanidine in the blood of each of these dogs before parathyroidectomy but an increased concentration at the time of the onset of tetany. Since the antagonism between the physiological actions of guanidine and calcium has been repeatedly recognized (6) (9) (4) (13) the occurrence of increased guanidine even inconstantly cannot be ignored in a condition in which the calcium tends to be deficient. It seems desirable, therefore, to investigate further the conditions favoring the occurrence of increased guanidine in parathyroid tetany.

Minot and Dodd (14) have shown that the guanidine content of the blood increases during rapid dehydration. The increased concentrations of guanidine which were observed in some instances following parathyroidectomy are of the same order of magnitude as those observed by Minot and Dodd following dehydration. Furthermore the frequent failure on the part of parathyroidectomized dogs to drink and retain water together with the severe panting and salivation which accompany attacks of tetany are conducive to dehydration. Since previous investigations of guanidine concentration in parathyroid tetany have failed to take into account the factor of dehydration we have endeavored to determine to what extent this factor may account for the incidental occurrence of hyperguanidinemia following removal of the parathyroid glands.

EXPERIMENTAL. The object of the experiments reported here is to show the effect produced by changes in water balance on the concentration of guanidine bases in the blood of parathyroidectomized dogs and of normal controls. A total of 68 dogs have been used in this investigation. The animals were thyroparathyroidectomized under ether anesthesia. All the subjects were kept without food during the experimental observations. The fluid administration and environmental temperatures were varied, as will be described below, for the various groups of experimental subjects and their controls. The concentrations of guanidine and non-protein nitrogen were determined in the whole blood and those of calcium, total protein, and inorganic phosphates in the serum. The electrical excitability and environmental and body temperatures were carefully followed. The guanidine concentrations were determined by the colorimetric technique described in detail by Minot and Dodd (14) which is a modification of the method of Major and Weber (11). The usual range of guanidine concentration found in the blood of normal dogs by this method is from 0.35 to 0.45 mgm. per 100 cc. Since it is known that guanidine determinations made by this method on markedly uremic bloods are unreliable the non-protein nitrogen values were obtained to serve as a guide for the interpretation of our guanidine figures. The non-protein

nitrogen concentrations in the blood of both the control and parathyroidectomized animals were all below the levels which appreciably influence the determination of guanidine. The parathyroidectomized dogs all showed a decrease in serum calcium and increase in inorganic phosphates with the onset of tetany. The changes in electrical excitability were also typical. Only the data which have a direct bearing upon our immediate problem are presented here.



The major portion of our experimental work is described under five headings and the effects on the guanidine concentrations in the blood are presented in figures 1 to 5. The dogs in all groups were kept without food for 12 to 16 hours and a preliminary sample of blood was drawn for chemical study before operation. Immediately following operation the parathyroidectomized dogs and controls were placed on the experimental regimes described below. At the onset of tetany in the parathyroidectomized animals a second sample of blood was taken from them and their controls. As tetany usually developed within 24 to 48 hours following operation the period of dehydration was in no case very long.

I. *Dogs kept at temperatures of 18° to 23°C. with water ad libitum.* At this environmental temperature there is little tendency to dehydration before the onset of tetany. Although failure to drink water is, as has been said, a common prodromal sign of tetany, there is usually a period of from 12 to 24 hours following operation when parathyroidectomized dogs take water normally. Thus when water is available there is only a relatively short period of abnormally low fluid intake. As may be seen from figure 1, neither the control nor parathyroidectomized animals show any significant rise in guanidine concentration in the blood. This observation is in accord with our previous reports that most dogs with parathyroid tetany studied in cool weather show no hyperguanidinemia.

II. *Dogs kept at temperatures of 18° to 23°C., no water available.* The opportunity for dehydration was increased in these experiments by withholding all water following operation. The fluid lost by the animals was approximately the same as in group 1. Again the animals with the parathyroids removed are similar to the controls but all show a slight or moderate rise in guanidine concentration. The difference in the duration of the experiments which were all terminated at the onset of tetany resulted in varying degrees of dehydration which explains the variability in degree of guanidine increase in different individuals in both normal and control groups. The results are shown in figure 2.

III. *Dogs kept at temperatures of 30° to 36°C., no water available.* All the animals in this group became markedly dehydrated. Their eyes were sunken and their noses dry. They passed little or no urine and the concentration of serum protein increased slightly in the more prolonged experiments. The control and parathyroidectomized dogs were subjected to the same degree of dehydration and showed similar increases in guanidine. It was noted that animals with a large amount of subcutaneous tissue could withstand the same lack of water and show a less marked rise in guanidine than that which occurred in thin emaciated subjects. This difference was as marked in the controls as in the dogs with the parathyroid glands removed. The results are shown in figure 3. The higher concentrations of guanidine reached in these experiments are at levels which commonly cause symptoms of intoxication. Most of the dogs were markedly depressed. In some instances fibrillary tremors in the muscles appeared in the parathyroidectomized animals before the concentration of calcium in the blood had fallen significantly. The influence of these higher concentrations of guanidine on tetany will be more fully discussed in a later paper.

IV. *Dogs kept at temperatures of 30° to 36°C. Water ad libitum.* In this group there is a great difference in the degrees of dehydration produced in the control and parathyroidectomized animals and marked discrepancies in the increase in guanidine appear even among the operated

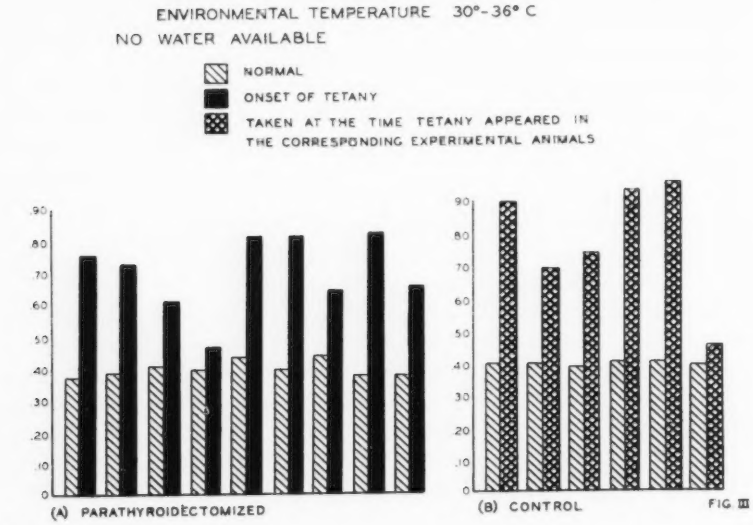


FIG. III

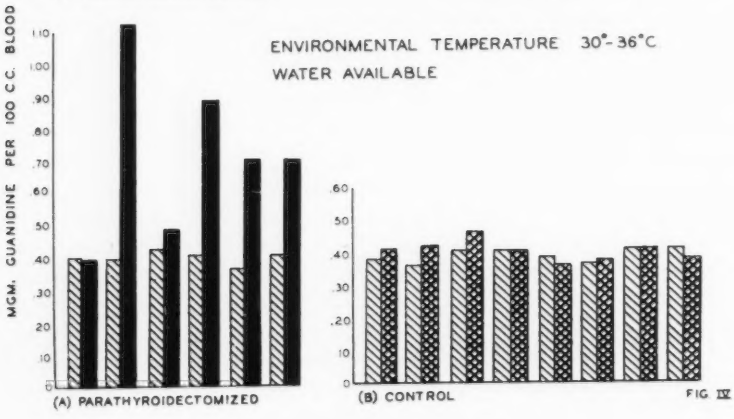


FIG. IV

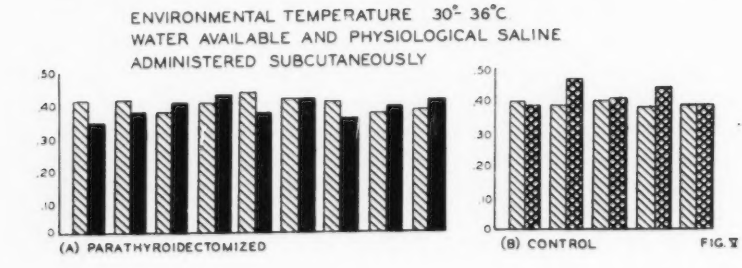


FIG. V

animals. The control dogs all drank water very freely and all of them had guanidine concentrations which are within normal limits. Some of the animals took water well following parathyroidectomy. For example, in the experiment presented in the first column in figure 4 the dog drank more water during the interval between operation and the onset of tetany than was taken by some of the control animals. This dog showed no increase in guanidine and developed only very mild tetany. In contrast to this result is that obtained in the experiment presented in the second column of the same figure. This animal failed to take water and the second sample of blood was not obtained until tetany had been present for some time. Here fluid loss due to prolonged panting and profuse salivation together with the failure to drink water resulted in severe dehydration with a very marked increase in guanidine in the blood. As a rule if the onset of tetany is delayed in subjects which fail to drink water there is marked dehydration accompanied by a considerable increase in guanidine. The results in this group presented in figure 4 illustrate well the inconstant findings to be expected when guanidine determinations are made in hot weather on the blood of dogs in parathyroid tetany when no particular attention is paid to the fluid intake. The subjects showing a marked increase in guanidine are in accord with our earlier positive results obtained in the summer months (2).

V. *Dogs kept at temperatures of 30° to 36°C. Water ad libitum, saline (0.9 per cent Na Cl solution) administered subcutaneously.* Dehydration was prevented in the animals of this group not only by having water available but also by the parenteral administration of fluid. Because of frequent vomiting following the administration of water by stomach tube, fluid in the form of 0.9 per cent sodium chloride solution was given subcutaneously in amounts adequate for each animal. Under uniform conditions of adequate fluid intake neither the control nor parathyroidectomized dogs showed an increase in guanidine in spite of the high environmental temperature. These experiments indicate that when the factor of dehydration is ruled out, uniformly normal concentrations of guanidine will be found in the blood of fasting parathyroidectomized dogs, regardless of the environmental temperature. The results obtained in this group are presented in figure 5.

Discussion. Taken together, these experiments show that the variabilities in the guanidine content of the blood of dogs in the early stages of parathyroid tetany can be explained largely on the basis of differing degrees of dehydration. The similarity between the results obtained in control and parathyroidectomized animals kept under the same environmental conditions indicates clearly that the removal of the parathyroid glands has in itself no influence on the concentration of guanidine bases in the blood. The contradictory reports in the literature in regard to the

occurrence of hyperguanidinemia in parathyroid tetany may very likely be due to differences in dehydration. The positive findings of some investigators may be associated with failure of the experimental subjects to take adequate amounts of fluid or to a combination of this factor with increased fluid loss due to hot weather or violent panting and profuse salivation in prolonged attacks of tetany.

SUMMARY AND CONCLUSIONS

1. Further evidence has been presented that hyperguanidinemia occurs in some but not in all parathyroidectomized dogs.

2. In fasting animals dehydration is the principal factor which determines whether or not an increase in guanidine occurs.

3. Similar degrees of dehydration produce similar increases in guanidine in the blood of both normal and parathyroidectomized dogs.

4. Since certain symptoms following removal of the parathyroid glands favor dehydration, a high environmental temperature is more conducive to hyperguanidinemia in parathyroidectomized dogs than in normal dogs unless measures are taken to insure an adequate fluid intake.

5. Parathyroid deficiency has in itself no influence on the concentration of guanidine in the blood.

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THE RATE OF OXYGEN UTILIZATION BY RAT KIDNEYS AT DIFFERENT RATES OF UREA EXCRETION

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The object of these experiments was to determine the relation between the work of the kidney and its rate of heat production. It was felt particularly desirable to try to obtain such data for rat kidney since so much is known of the compensatory hypertrophy occurring in the rat kidney due to altered rates of urea excretion. The method used for measuring the heat production, while it offers distinct disadvantages which have been noted by Cushny and others, is particularly suitable for use with these small animals, since by accumulating many observations on different rats the average error, arising from the nature of the method, becomes insignificant. In brief, the method is that of estimating renal oxygen consumption by difference between the rate of intact rats and of the same animals after clamping the renal pedicle. The period of observation of rate of oxygen absorption of intact rats, the time required for clamping the renal pedicle, and the period of observation of the rate of oxygen absorption afterward required a total of less than ten minutes per rat, and usually of less than seven minutes per rat.

METHOD. Male white rats, of about 200 grams, were used. The right kidney was removed, and after allowing three weeks for renal hypertrophy the animals were separated into two groups. The weight and age of the rats in the two groups were the same. One group was put on 10 per cent dextrose, the other on 10 per cent dextrose plus 10 per cent urea for 24 hours before the observations on urea excretion or renal metabolism. Four pairs of rats were used to determine the rate of urea excretion. Renal oxygen utilization rates were measured satisfactorily in 32 pairs of rats. Rates of urine and urea excretion were determined before and after anesthesia (0.1 cc. 5 per cent pentobarbital solution per 100 gm.). The blood urea was determined at the end of the observation period. Work of urea excretion was calculated with the formula: $W = RT\mathrm{U}\left(\log \frac{Cu}{Cb} - \frac{Cu\text{-}Cb}{Cu}\right)$, where U is amount urea excreted (in mols), Cu, concentration of urea in

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urine, *Cb* concentration of urea in blood. For work in calories, at 37°C., $RT = 620$. Oxygen absorption rate was measured by the method previously described (1), but the spirometer and CO_2 absorption chamber were entirely water-jacketed, and the CO_2 absorption chamber was a thin walled copper tube, 1.4 cm. in diameter. With this modification the temperature change during observations was diminished, varying less than 0.2°C. during the time required for the observations on each animal. The total volume of gas in the spirometer device was 50 cc. when the soda lime container was full; the calibration covered 30 cc. The caloric value of oxygen, in animals on a high carbohydrate diet, was taken as 5 small calories per cubic centimeter of O_2.

It was found that the body temperature of the rats must be kept constant by applying external heat from electric pads or light bulbs, anesthesia must be deep and constant, and respiration easy and regular in order to obtain constant readings of rate of oxygen use. Fifteen minutes after

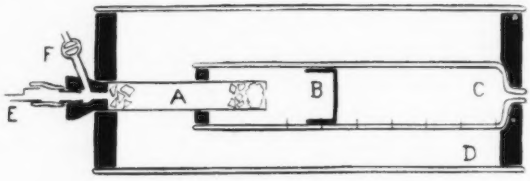

Fig. 1. Spirometer for determining rate of oxygen consumption of anesthetized rats. *A* is the tube of soda-lime; *B*, the bakelite "float;" *C*, 30-cc. Luer syringe; *D*, glass-walled water jacket; *E*, tracheal cannula; *F*, stop-clock in the O_2 supply tube.

intraperitoneal injection of pentobarbital, anesthesia became quite satisfactory, but occasionally, in rats on dextrose solution, pulmonary edema and tracheal obstruction invalidated any attempt at accurate estimate of metabolic rate. The rats on urea and dextrose solution required less anesthetic, reached a basal level more quickly, and had drier tissues, with no tendency to pulmonary edema. Under this level of pentobarbital anesthesia renal secretion was maintained for an hour; with additional doses of 0.05 cc. per 100 grams, whenever anesthesia began to wear off (usually at 40–60 min. intervals) urine secretion could be studied satisfactorily for 4 hours. After the rate of O_2 absorption for the entire rat was determined, an incision was made in the left flank and the pedicle of the remaining kidney clamped off. Rate of oxygen absorption was again determined, and the difference in rate, from that of the intact rat, was taken as the amount of O_2 per minute utilized by the kidney. Such a method of determining oxygen consumption of an organ, especially when the amount used by the organ is only 5 to 10 per cent of that used by the intact animal, can not pretend to great accuracy. However, by using a relatively large

group of animals the error of the method is diminished and the difference in the average rates becomes statistically significant. The enormous variations in the rate and concentration of urea excretion which are obtained under the above conditions in the rat are a great advantage in relating work to heat production.

RESULTS. The results of the observations are summarized in tables 1 and 2. There were very great differences in the amount of urea excreted and in the rate of work done in excreting urea by the rats in the two groups.

TABLE 1

	RAT WEIGHT	O_2 PER RAT PER MINUTE	KIDNEY WEIGHT	KIDNEY O_2 PER GM. PER MINUTE	RENAL O_2, PER CENT TOTAL	RATIO RENAL O_2 PER GM. TOTAL O_2 PER GM.	RENAL METABOLISM CAL. PER GM. PER MINUTE
	gm.	*cc.*	*gm.*	*cc.*	*per cent*		*cal.*
Rats given dextrose solution	221	3 99	1 13	0 185	5 2	10 3	0 92
Rats given urea solution	210	3 90	1 20	0 227	7 0	12 2	1 13
Per cent difference due to urea	−5 0	−2 1	+6 2	+22 6			+22 6%

TABLE 2

	UREA EXCRETION BEFORE ANESTHESIA PER RAT	URINE VOLUME BEFORE ANESTHESIA PER RAT	UREA EXCRETION (ANESTHETIZED) PER GM. KIDNEY	URINE EXCRETION (ANESTHETIZED) PER GM. KIDNEY	BLOOD UREA	WORK OF UREA EXCRETION CAL. PER GM. PER MIN	EFFICIENCY OF UREA EXCRETION
	mgm./min.	*cc./min.*	*mgm./min.*	*cc./min.*	*mgm. per cent*	*cal.*	*per cent*
Rats given dextrose solution	0.13	0.133	0.07	0.037	28 5	0.00075	0 08
Rats given urea solution	1 98	0.03	1.4	0.014	221	0.043	3 8
Per cent difference due to urea	+1520%	−78%	+1900%	−42%	+658%	+5620%	+4780%

However, the difference in renal oxygen metabolism of rats in the two groups was small. The mean value, for rats on dextrose, was 0.185 cc. per gram per minute, the standard deviation was 0.041; for the rats on urea solution, the mean was 0.227 cc., the standard deviation 0.055. The difference of the means, 0.042, was 5.1 times the probable error of the means, 0.0082. From these figures one may conclude that the rate of renal oxygen absorption is higher in the rats on urea solution than in those on dextrose; but that the rate of renal metabolism, due to high urea intake is only 23 per cent (± 5 per cent) greater in the rats with high urea intake than in those receiving dextrose solution only.

Under the conditions of these experiments an increase of only 22.6 per cent in rate of renal oxygen absorption is produced by a fifty-six fold increase in the rate of work done in excreting urea. An increase of 0.21 calorie per gram per minute energy liberation by the kidney accompanies an increase of 0.042 calorie work done in excreting urea.

Borsook and Winegarden (3), (4) recently summarized the literature on renal work and its cost and added some observations on the total heat production of normal men before and after urea ingestion. They concluded that the heat production by the kidneys was very definitely augmented by

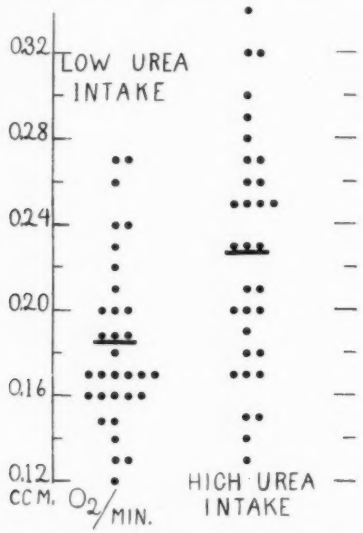

Fig. 2. The distribution of data on renal oxygen consumption. The rates per gram of tissue per minute of individual observations are given by the dots, the means for each group by heavy bars.

increasing the rate of urea excretion. On the other hand, Rhoads and Van Slyke (5) state that no increase in renal metabolism occurs with augmented rates of urea elimination by dogs. Glaser, Laszlo and Schurmeyer (5), (6), using dogs, found no regular correlation between renal oxygen metabolism and the rate at which the kidneys performed work in overcoming osmotic pressure. In the rat, the O_2 absorption rate during excretion of hypotonic urine, low in urea, as compared with that during excretion of urine containing 10 per cent urea, indicates a rise in renal heat production with increase in osmotic work. But the rise in heat production is very small as compared with the rise in osmotic work (22.6 per cent increase in O_2 need, 5620 per cent increase in work), and indicates that, in

the rat, less than 5 per cent of the specific dynamic action of protein can be due to the extra heat following extra urea excretion. It is possible that the figure for basal renal activity, obtained in rats secreting hypotonic urine, is higher than that of rats with lower rates of water elimination. Even making allowance for the work done by the kidney in secreting hypotonic urine, and the relative inefficiency of organs working under a load less than the normal, it is almost certain that the rat kidney never manifests an increase in O_2 requirement in any way parallel to the increase in osmotic work.

SUMMARY. From indirect measurement of rate of renal oxygen metabolism in rats anesthetized with pentobarbital it was found that the increase of O_2 requirement, due to a fifty-six fold increase in osmotic work done in urea elimination, was only 22.6 per cent. The extra heat formed in the kidney excreting very large amounts of urea, as compared with the heat formation during very low rates of urea excretion, was five times as great as the caloric equivalent of the extra osmotic work of urea elimination.

CONCLUSION

The extra work imposed on the rat kidney by a rise in urea intake is accompanied by the liberation of extra heat in the kidney. This increase is so slight that the extra energy required to excrete the urea derived from amino-acid would represent only 1 to 4 per cent of the total specific dynamic action.

The total increase in oxygen absorption by the kidney is five times as great as the caloric equivalent of the work done in secreting extra urea, but the percentage increase in oxygen absorption is insignificant in comparison with the per cent increase in osmotic work.

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